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PATENT & TRADEMARK OFFICE

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FULBRIGHT & JAWORSKI L.L.P.

A REGISTERED LIMITED LIABILITY PARTNERSHIP
600 CONGRESS AVENUE, SUITE 2400
AUSTIN, TEXAS 78701-3271
WWW.FULBRIGHT.COM

RHANSON@FULBRIGHT.COM
DIRECT DIAL: (512) 536-3085

TELEPHONE: (512) 474-5201
FACSIMILE: (512) 536-4598

May 30, 2006

CERTIFICATE OF MAILING
37 C.F.R. 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date below:

May 30, 2006
Date

Robert E. Hanson

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Re: *SN 10/820,222 "PLANTS AND SEEDS OF CORN VARIETY I113752" by Daniel J. Lubich*
Our Ref. DEKA:341US; Client Ref. 34-63 (51698)

Commissioner:

Enclosed for filing in the above-referenced patent application is:

1. An Appeal Brief;
2. A Petition for Extension of Time;
3. A check for $620.00 to cover the Appeal Brief filing fee and the one-month extension fee; and
4. A return postcard to acknowledge receipt of these materials. Please date stamp and mail this postcard.

If the check is inadvertently omitted, or the amount is insufficient, or should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed materials, or should an overpayment be included herein, the Commissioner is authorized to deduct or credit said fees from or to Fulbright & Jaworski L.L.P. Account No.: 50-1212/DEKA:341US.

Respectfully submitted,

Robert E. Hanson
Reg. No. 42,628

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Enclosure

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Daniel J. Lubich	Group Art Unit: 1638
Serial No.: 10/820,222	Examiner: Fox, D.
Filed: April 6, 2004	Atty. Dkt. No.: DEKA:341US
For: PLANTS AND SEEDS OF CORN VARIETY I113752	

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BRIEF ON APPEAL

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Daniel J. Lubich

Serial No.: 10/820,222

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For: PLANTS AND SEEDS OF CORN VARIETY I113752

Group Art Unit: 1638

Examiner: Fox, D.

Atty. Dkt. No.: DEKA:341US

BRIEF ON APPEAL

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Appellants hereby submit an original and two copies of this Appeal Brief. The date for filing this Brief is May 30, 2006 based on the Petition for Extension of Time of One-Month filed herewith. The extension fee and fees for filing this Appeal Brief are attached. However, should any additional fees become due under 37 C.F.R. §§ 1.16 to 1.21 for any reason relating to the enclosed materials, or should an overpayment be made, the Commissioner is authorized to deduct or credit said fees from or to Fulbright & Jaworski Deposit Account No. 50-1212/DEKA:341US.

Please date stamp and return the attached postcard as evidence of receipt.

I. REAL PARTY IN INTEREST

The Real Party in Interest is Monsanto Company, the parent company of assignee Monsanto Technology LLC.

II. RELATED APPEALS AND INTERFERENCES

There are no appeals or interferences for related cases. However, the following appeals have been decided by the Board and involve issues substantially identical to those presented in the current appeal: Appeal Nos: 2004-1503 (Ser No. 09/606,808), 2004-1506 (Ser. No. 09/788,334), 2004-1968 (Ser. No. 10/000,311), 2004-2317 (Ser. No. 09/771,938), 2004-2343 (Ser. No. 09/772,520); and 2005-0396 (Ser. No. 10/077,589). As with the current case, these cases relate to corn plant varieties, share the same Real Party in Interest, and raise many of the same issues as the current appeal, but are directed to different corn plant varieties.

Appeals have also been filed by the Real Party in Interest, but have not yet been decided by the Board, in the following cases which involve corn or soybean plant varieties and present substantially the same issues as the current appeal: U.S. Patent Application Ser. Nos. 09/702,782, 09/708,649, 10/310,639, 10/310,661, 10/310,696, 10/310,708, 10/310,716, 10/350,148, 10/350,156, 10/350,293, 10/352,377, 10/352,386, 10/369,190, 10/369,212, 10/370,027, 10/370,040, 10/370,274, 10/370,275, 10/370,287, 10/384,174, 10/384,358, 10/384,368, 10/384,393, 10/819,093, 10/819,104 and 10/820,222.

III. STATUS OF CLAIMS

Claims 1-24 were filed with the case and are currently pending. Claims 1-5, 7-10 and 12-14 have been allowed and claims 6, 11 and 15-24 finally rejected. A copy of the appealed claims is provided in the Claims Appendix.

IV. STATUS OF AMENDMENTS

No amendments were made subsequent to the Final Office Action.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The claimed invention at issue relates to male sterile and locus converted plants of variety I113752 and plants possessing the characteristics of variety I113752. Specification from page 6, line 6 to page 7, line 16. The invention further relates to methods of breeding plants of variety I113752 with other corn plants, and F1 hybrid plants produced thereby. Specification from page 7, line 17 to page 9, line 14.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

(A) Were claims 6, 11, 15-16 and 20 properly rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out the subject matter which Appellants regard as the invention?

(B) Were claims 6, 11, and 15-24 properly rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement?

(C) Were claims 6, 11, and 15-24 properly rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement?

(D) Was claim 11 properly rejected under 35 U.S.C. §102 as being anticipated?

VII. ARGUMENT

A. The Issues On Appeal Have Been Decided in Appellants' Favor by The Board

On March 31, 2005 the Board of Patent Appeals decided six substantially similar appeals brought by the Real Party in Interest: Appeal Nos: 2004-1503 (Ser No. 09/606,808), 2004-1506 (Ser. No. 09/788,334), 2004-1968 (Ser. No. 10/000,311), 2004-2317 (Ser. No. 09/771,938), 2004-2343 (Ser. No. 09/772,520); and 2005-0396 (Ser. No. 10/077,589) (collectively "the corn variety appeals"). The claims at issue in these cases were substantially identical in scope to those of the current case. Each of the prior cases and the current case was examined in the same art unit and included essentially identical written description, enablement and indefiniteness rejections.

In the prior appeals all rejections were reversed. The Examiner here has refused to give deference to the Board decisions because they were not published and designated binding precedent. However, cases presenting the same issues on the same operative facts should be examined consistently. All similar rejections raised in the current appeal should therefore be reversed for the same reasons presented by the Board in the corn variety appeals, as explained below. In addition, Appellants respectfully request that any subsequent decision on this case be designated as binding precedent so that additional expenses and waste of resources incurred by the Office and Appellants can be avoided in future cases.

B. The Claims Are Definite Under 35 U.S.C. §112, Second Paragraph

1. Rejection of claim 6

The Action asserts that claim 6 is indefinite for its recitation of "corn plant of claim 2, further comprising a nuclear or cytoplasmic gene conferring male sterility" as it is asserted that this is inconsistent with claim 2.

a) The rejection is without merit

The current claim (1) contains a reference to the parent claims from which it depends, (2) contain a further limitation of the main claim, and (3) incorporates all elements of the claim from which it depends. The meaning of the claim and relationship with the parent claims is thus not unclear and the claim is in proper dependent form pursuant to 37 C.F.R. §1.75(c). The rejection is therefore without merit.

b) The Board has decided the issue in favor of Appellants

Applicants note that this rejection has already been decided in Applicants favor by the Board of Patent Appeals in a substantively identically worded claim in an application presenting the same operative facts. In particular, in Appeal No. 2005-0396, Application No. 10/077,589, the same rejection was made and reversed. In that case claim 16 read as follows "16. The corn plant of claim 15, further comprising a nuclear or cytoplasmic gene conferring male sterility." The Examiner rejected the claim on the same grounds as here, namely that the claim was inconsistent with the claim from which it depended. The Board reversed the rejection, stating that:

> For example, claim 16 reads on a corn plant capable of expressing all the physiological and morphological characteristics of the corn variety I180580, further comprising a nuclear or cytoplasmic gene conferring male sterility. In our opinion, the claims reasonably apprise those of skill in the art of their scope. *Amgen*, As set forth in *Shatterproof Glass Corp. v. Libbey-Owens Ford Co.*, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir. 1985), '[i]f the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the

> utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more.' Accordingly we reverse the rejection of claims 16 and 27-30 under 35 U.S.C.§ 112, second paragraph.

Exhibit A at p. 12

As explained by the Board, the claim is fully definite. In view of the Board's decision on this issue, removal of the rejection is respectfully requested.

2. Rejection of claim 11

The Action asserts that in claim 11 "capable of" is indefinite because it is unclear whether the plant does or does not express the physiological or morphological traits of the claimed variety.

a) The rejection is without merit

There is nothing indefinite about the current claim as the terms used have a clear meaning in the art and claim breadth is not indefiniteness. One of skill in the art would understand whether a corn plant is capable of expressing all of the traits of the claimed corn plant because Appellants have provided the corn plant by way of a proffered biological deposit with the ATCC and have described the characteristics of this plant. One of skill in the art would therefore readily ascertain whether a plant is capable of expressing all of the traits of the claimed variety based on direct comparisons under similar growing conditions. Because this standard is readily ascertainable, the use of the limitation in the claims is not indefinite. Reversal of the rejection is therefore respectfully requested.

b) The Board has decided the issue in favor of Appellants

The same rejection was made and reversed in Appeal No. 2005-0396. In that case claim 20 read as follows "20. A corn plant regenerated from the tissue culture of claim 17, wherein the corn plant is capable of expressing all of the physiological and morphological characteristics of the corn variety designated I180580, wherein a sample of the seed of the corn variety I180580

was deposited under ATCC Accession No. PTA-3224." The Examiner ejected the claim on the same grounds as here, namely that because the claims use the term "capable" the claims do "not make clear if the plant actually expresses the traits, or when or under what conditions the traits are expressed." The Board reversed, explaining that

> To address the examiner's concerns, we find it sufficient to state that if a plant has the capacity to express the claimed characteristics it meets the requirement of the claim regarding 'capable of,' notwithstanding that due to a particular phase of the life cycle the plant is not currently expressing a particular characteristic. Alternatively, if a plant is incapable of expressing the claimed characteristics at any phase of the life cycle, because it lacks, for example, the 'transcription factor' required for expression - such a plant would not meet the requirement of the claim regarding "capable of."
>
> Here, we find the examiner's extremely technical criticism to be a departure from the legally correct standard of considering the claimed invention from the perspective of one possessing ordinary skill in the art. In our opinion, a person of ordinary skill in the art would understand what is claimed. *Amgen Inc. v. Chugai Pharmaceutical Co., Ltd.*, 927 F.2d 1200, 1217, 18 USPQ2d 1016, 1030 (Fed. Cir. 1991). We find the same to be true for the phrase 'capable of' as set forth in claims 17 and 20.

Decision at p. 12.

In view of the foregoing, reversal of the rejection is respectfully requested.

3. Rejection of claim 15

The Action rejects claim 15 as confusing for recitation of "corn plant of claim 2, further comprising a transgene" which is said to be inconsistent with claim 2.

a) The rejection is without merit

The current claim (1) contains a reference to the parent claims from which it depends, (2) contain a further limitation of the main claim, and (3) incorporates all elements of the claim from which it depends. The meaning of the claim and relationship with the parent claims is thus not unclear and the claim is in proper dependent form pursuant to 37 C.F.R. §1.75(c). The rejection is therefore without merit.

b) The Board has decided the issue in favor of Appellants

The same issue was considered and decided in Applicants favor in Appeal No. 2005-0396. In that case, claim 27 read as follows "27. The corn plant of claim 5, further defined as having a genome comprising a single locus conversion." The Examiner rejected the claim on the same grounds as here, namely that "the examiner finds it unclear whether the plant set forth in claim 27 has all the characteristics of the plant set forth in claim 5, from which claim 27 depends." **Exhibit A** at p. 12.

In response to the rejection of this and another claim (16) the Appellants noted that the claims simply add a further limitation to the claims from which they depend. The Board reversed the examiner and explicitly agreed with Appellants, stating that:

> For example, claim 16 reads on a corn plant capable of expressing all the physiological and morphological characteristics of the corn variety I180580, further comprising a nuclear or cytoplasmic gene conferring male sterility. In our opinion, the claims reasonably apprise those of skill in the art of their scope. *Amgen*, As set forth in *Shatterproof Glass Corp. v. Libbey-Owens Ford Co.*, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir. 1985), '[i]f the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more.'

Exhibit A at p. 12.

The same issue as presented here has therefore been resolved by the Board in Applicants favor. Removal of the rejection is thus respectfully requested.

4. Rejection of claim 20

The Action rejects claim 20 as indefinite for recitation of "genetic locus was stably inserted into a corn genome" on the basis that it is unclear whether the locus is inserted into the plant of claim 20 or another plant.

a) The rejection is without merit

The locus referred to in the claim may or may not have been directly inserted into the genome of the claimed plant and this does not render the claim indefinite. For example, the locus may have been inserted into a parent of the claimed variety that has been self pollinated to produce the claimed plant. Such a plant would therefore not have been directly transformed with the locus but the locus would have been created by transformation. That is, loci stably inserted into a corn genome are stably inherited and thus the locus need not have been inserted directly into the genome of a given single plant of corn variety. As such, the metes and bounds of the claim are clear and the claim is not indefinite.

b) The Board has decided the issue in favor of Appellants

The position taken has already been rejected by the Board of Patent Appeals in Appeal No. 2005-0396. In that case, claim 28 read as follows "28. The corn plant of claim 27, wherein the single locus was stably inserted into a corn genome by transformation." The Examiner rejected the claim on the same grounds as here, namely that "the recitation does not make clear if the genome is that of [the claimed variety] or that of a different corn plant." **Exhibit A** at p. 13. The Board reversed, explaining that

> we agree with appellant (Brief, page 9) '[t]he single locus referred to in claim 28 may or may not have been directly inserted into the genome of the claimed plant.' As we understand the claim, and arguments of record, claim 28 presents two possibilities: (1) the single locus is directly inserted into the claimed plant and nothing further need be done; or (2) the single locus is directly inserted into a different plant, which is then used to transfer the single locus to the claimed plant through use of the plant breeding technique known as backcrossing.
>
> In our opinion, the claim reasonably apprises those of skill in the art of its scope. *Amgen.* Accordingly, we reverse the rejection of claim 28 under 35

Exhibit A at p. 13-14.

The issue has therefore been resolved by the Board. Removal of the rejection is thus respectfully requested.

C. The Claims Satisfy the Written Description Requirement of 35 U.S.C. §112, First Paragraph

The Examiner rejected claims 6, 11 and 15-24 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to convey that Appellants were in possession of the claimed invention. For example, it was asserted that plant of the claimed variety comprising a male sterility gene or locus conversion, F1 hybrid progeny of the claimed variety and methods of plant breeding comprising use of the claimed variety as starting material lack written description. The rejections should be reversed as set forth below.

1. The Issues Raised Have Been Decided by The Board in Appellants' Favor

All of the rejections made have specifically been considered and reversed by the Board of Patent Appeals on the same operative facts as presented in the current case. The rejections should be reversed by the Board on the same basis, as explained below.

In the corn variety appeals, for example, rejections were raised based on an alleged lack of written description for F1 hybrid plants. Specifically, it was alleged that the F1 hybrid plants only have as half of their genome the genome of the parent plant of interest and the remaining portion was not described, and thus written description is lacking, despite the fact that no particular second hybrid plant is required by the method. See Board Decision in Appeal No. 2005-0396 at p. 17. The Board rejected this reasoning, noting that, as here, the claims require no particular second parent of the F1 hybrid and the Examiner had already acknowledged written description for the variety of interest. The Board thus held that "there can be no doubt that the

specification provides and adequate written description of this corn variety." *Id.* at p. 18. As explained by the Board, the purpose of the written description requirement is to "ensure that the right of the scope to exclude, as set forth in the claims does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification." *Id.* Here, as in the corn variety appeals, the claimed F1 hybrid plants must have the admittedly described variety of interest as one parent, and thus the claims do not overreach the scope of the inventor's contribution and are fully described.

The dispositive issue was the Board's rejection of the notion that Appellants must define every possible second hybrid parent plant and the morphological characteristics of the progeny thereof. In particular, the Board stated that it "disagree[d] with the examiner's conclusion (id.) that '[t]he fact that any hybrid plant will inherit half of its alleles from [the variety of interest] then does not provide sufficient description of the morphological and physiological characteristics expressed by the claimed hybrid plants." *Id.*

Methods of plant breeding are similarly described. Essentially identical written description rejections were raised by the examiner, for example, in Appeal No. 2005-0396 of claims drawn to a method of breeding corn plants comprising use of the variety of interest as starting material. In the appeal the Examiner argued that written description is lacking because the intermediate plants at each step of the method allegedly must be described to satisfy written description and that such plants had not been defined, regardless of the fact that the only starting material required by the claims was the variety of interest, which was admittedly fully defined. See *Id.* at p. 12-15. The Board rejected this reasoning, noting that, given the acknowledgement of written description for the variety of interest, appellants were also in possession of a method of using that plant for crossing with any other plant to produce an inbred plant as set forth in the

claims. The Board thus concluded that appellant established with reasonable clarity that they were in possession of the invention. In the context of methods of introducing transgenes, the Board noted that no evidence was provided to support the rejections or inadequacy of written description for the claims. The Board thus reversed the written description rejections.

The same issues have been presented here on claims of an essentially identical scope and thus the rejections should be reversed on the same basis.

2. The Rejections Are Without Merit

a) Male Sterile and Locus Converted Plants Are Described

The Examiner rejected claims directed to plants of variety I113752 further comprising a nuclear or cytoplasmic gene conferring male sterility or a locus conversion of this variety, as lacking written description under 35 U.S.C. §112, first paragraph. For example, the Examiner appeared to assert that: (1) the characteristics of the claimed plants are unpredictable and/or not described, (2) the claims encompass genes that have yet to be discovered, and (3) the sequences and/or sources for the numerous examples of loci disclosed in the application have not been described.

(1) The claimed subject matter is not unpredictable and is described

With regard to the first point, it is noted that a "converted (conversion) plant" is defined in the specification as "Plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to one or more new trait conferred by the genetic locus transferred into the inbred via the backcrossing technique." Therefore, the claimed plants comprising a locus conversion possess "essentially all of the desired morphological and physiological characteristics of [the locus converted plant]". Similarly, the claims are directed to

a corn plant that is capable of expressing all the physiological and morphological characteristics of the corn variety I113752 which is further defined as comprising a nuclear or cytoplasmic gene conferring male sterility. The Examiner's comments with regard to various allegedly unknown characteristics are thus outside the scope of the claims. With regard to the claimed subject matter, Appellants have more than adequately described such a plant that comprises essentially all of the desired morphological and physiological characteristics of corn plant I113752 by way of the description and proffered deposit of I113752 alone, not to mention the additional description provided. To hold otherwise would be to limit Appellants to that subject matter described *ipsis verbis* in the specification. This position is expressly contradictory to Federal Circuit precedent. *In re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989) (stating that the written description requirement does not require an applicant to "describe exactly the subject matter claimed, [instead] the description must clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed" (citations omitted)) .

(2) The Examiner has applied the written description requirement with respect to unclaimed subject matter

With respect to the Examiner's apparent allegation that the claims encompass genes that are not described or have yet to be discovered, it is noted that Appellants ***do not claim genes***. The claimed subject matter is the corn variety I113752 comprising a nuclear or cytoplasmic gene conferring male sterility or a locus conversion. Nuclear and cytoplasmic genes conferring male sterility and introduction of these genes into corn varieties have been well known for many years (see U.S. Patent No. 3,861,709; U.S. Patent No. 3,710,511; U.S. Patent No. 4,654,465; U.S. Patent No 5,625,132; U.S. Patent No. 4,727,219; U.S. Patent No. 5,530,191; U.S. Patent No. 5,689,041; U.S. Patent No. 5,741,684; and U.S. Patent No. 5,684,242). Further, any locus

conversion may be introduced into corn variety I113752 to produce locus conversions. The fact that a given gene could be isolated in the future and introduced as a locus conversion is irrelevant – the new gene is not claimed *per se*, a locus conversion of corn plant I113752 is claimed. Under the reasoning of the Examiner, essentially any claim could be read to encompass subject matter yet to be invented and therefore not be described. A claim to a corn plant transformed with a *Bacillus thuringiensis* gene would be invalid because it would encompass corn varieties yet to be discovered. A claim to a given gene operably linked to a regulatory element would be invalid because as yet to be isolated regulatory elements would be encompassed. Nearly any biotechnological invention could be viewed this way applying the Examiner's reasoning. However, it is not any given locus that is claimed, it is a corn plant of corn variety I113752 which comprises a converted locus that has been claimed.

(3) Appellants have disclosed numerous traits for a locus conversion and such traits were well known to those of skill in the art

The Examiner alleged that Appellants have not described the full genus of loci for preparation of converted plants. However, numerous such traits were described by Appellants and are known in the art. Among just the examples in the specification recited with a publication reference or patent number are the following (see specification at pages 29-34): genes conferring male sterility (U.S. Patent No. 3,861,709, U.S. Patent No. 3,710,511, U.S. Patent No. 4,654,465, U.S. Patent No 5,625,132, and U.S. Patent No. 4,727,219, incorporated by reference); male-sterility restorer genes (U.S. Patent Nos. 5,530,191, 5,689,041, 5,741,684, and 5,684,242, incorporated by reference); a herbicide resistant EPSPS mutation termed *aroA* (U.S. Patent 4,535,060); and a mutant maize gene encoding a protein with amino acid changes at residues 102 and 106 (PCT Publication WO 97/04103).

The locus traits are also described by way of PCT Application Publ. WO 95/06128, which was specifically incorporated by reference in the specification. Examples of some of the traits described in WO 95/06128, including the corresponding gene and any associated phenotype and publication reference given, are as follows:

the uidA gene from *E. Coli* encoding β-glucuronidase (GUS) (cells expressing *uidA* produce a blue color when given the appropriate substrate, Jefferson, R.A. 1987. *Plant Mol. Biol. Rep* 5: 387-405); the *bar* gene from *Streptomyces hygroscopicus* encoding phosphinothricin acetyltransferase (PAT) (cells expressing PAT are resistant to the herbicide Basta, White, J., Chang, S.-Y.P., Bibb, M.J., and Bibb, M.J. 1990. *Nucl. Ac. Research* 18: 1062); the *lux* gene from firefly encoding luciferase (cells expressing *lux* emit light under appropriate assay conditions, deWet, J.R., Wood, K.V., DeLuca, M., Helinski, D.R., Subramani, S. 1987. *Mol. Cell. Biol.* 7: 725-737); the *dhfr* gene from mouse encoding dihydrofolate reductase (DHFR) (cells expressing *dhfr* are resistant to methotrexate; Eichholtz, D.A., Rogers, S.G., Horsch, R.B., Klee, H.J., Hayford, M., Hoffman, N.L., Bradford, S.B., Fink, C., Flick, J., O'Connell, K.M., Frayley, R.T. 1987. *Somatic Cell Mol. Genet.* 13: 67-76); the *neo* gene from *E.Coli* encoding aminoglycoside phosphotransferase (APH) (cells expressing *neo* are resistant to the aminoglycoside antibiotics; Beck, E., Ludwig, G., Auerswald, E.A., Reiss, B., Schaller, H. 1982. *Gene* 19: 327-336); the *amp* gene from *E. Coli* encoding β-lactamase (cells expressing β-lactamase produce a chromogenic compound when given the appropriate substrate; Sutcliffe, J.G. 1978. *Proc. Nat. Acad. Sci.* USA 75: 3737-3741); the *xylE* gene from *Ps. putida* encoding catechol dihydroxygenase (cells expressing *xylE* produce a chromogenic compound when given the appropriate substrate; Zukowsky *et al.* 1983. *Proc. Nat. Acad. Sci.* USA 80: 1101-1105); the R,C1 and B genes from maize encode proteins that regulate anthocyanin biosynthesis in maize (Goff, S., Klein, T., Ruth, B., Fromm, M., Cone, K., Radicella, J., Chandler, V. 1990. *EMBO J.:* 2517-2522); the ALS gene from *Zea mays* encoding acetolactate synthase and mutated to confer resistance to sulfonylurea herbicides (cells expressing ALS are resistant to the herbicide; Gleen. Yang, L.Y., Gross, P.R., Chen, C.H., Lissis, M. 1992. *Plant Molecular Biology* 18: 1185-1187); the proteinase inhibitor II gene from potato and tomato (plants expressing the proteinase inhibitor II gene show increased resistance to insects; potato - Graham, J.S., Hall, G., Pearce, G., Ryan, C.A. 1986 *Mol. Cell. Biol.* 2: 1044-1051; tomato - Pearce, G., Strydom, D., Johnson, S., Ryan, C.A. 1991. *Science* 253: 895-898); the *Bt* gene from *Bacillus thuringensis* berliner 1715 encoding a protein that is toxic to insects (this gene is the coding sequence of *Bt* 884 modified in two regions for improved expression in plants; Vaeck, M., Reynaerts, A., Hofte, H., Jansens, S., DeBeuckeleer, M., Dean, C., Aeabeau, M., Van Montagu, M., and Leemans, J. 1987. *Nature* 328: 33-37); the *bxn* gene from *Klebsiella ozaeneae* encoding a nitrilase enzyme specific for the herbicide bromoxynil (cells expressing this gene are resistant to the herbicide bromoxynil; Stalker, D.m., McBride, K.E., and Malyj, L. *Science* 242: 419-422, 1988); the WGA-A gene encoding wheat germ agglutinin (expression of the WGA-A gene confers resistance to insects; Smith, J.J., Raikhel, N.V. 1989. *Plant Mol. Biology* 13: 601-603); the *dapA* gene from *E. coli* encoding dihydrodipicolinate synthase (expression of this gene in plant cells produces increased levels of free lysine; Richaud, F., Richaud, C., Rafet, P. and Patte, J.C. 1986. *J. Bacteriol.* 166: 297-300); the *Z10* gene encoding a 10kd zein storage

protein from maize (expression of this gene in cells alters the quantities of 10kD Zein in the cells; Kirihara, J.A., Hunsperger, J.P., Mahoney, W.C., and Messing, J. 1988. *Mol. Gen. Genet.* 211: 477-484); the Bt gene cloned from *Bacillus thuringensis* Kurstaki encoding a protein that is toxic to insects (the gene is the coding sequence of the cry IA(c) gene modified for improved expression in plants - plants expressing this gene are resistant to insects; Höfte, H. and Whiteley, H.R., 1989. *Microbiological Reviews.* 53: 242-255); the ALS gene from *Arabidopsis thaliana* encoding a sulfonylurea herbicide resistant acetolactate synthase enzyme (cells expressing this gene are resistant to the herbicide Gleen. Haughn, G.W., Smith, J., Mazur, B., and Somerville, C. 1988. *Mol. Gen. Genet.* 211: 266-271); the *dehl* gene from *Pseudomonas putida* encoding a dehalogenase enzyme (cells expressing this gene are resistant to the herbicide Dalapon; Buchanan-Wollaston, V., Snape, A., and Cannon, F. 1992. *Plant Cell Reports* 11: 627-631); the hygromycin phosphotransferase II gene from *E. coli* (expression of this gene in cells produces resistance to the antibiotic hygromycin. Waldron, C., Murphy, E.B., Roberts, J.L., Gustafson, G.D., Armour, S.L., and Malcolm, S.K. *Plant Molecular Biology* 5: 103-108, 1985); the *mtlD* gene cloned from *E. coli* (the gene encodes the enzyme mannitol-1-phosphate dehydrogenase; Lee and Saier, 1983. *J. of Bacteriol.* 153:685); the HVA-1 gene encoding a Late Embryogenesis Abundant (LEA) protein (the gene was isolated from barley; Dure, L., Crouch, M., Harada, J., Ho, T.-H. D. Mundy, J., Quatrano, R, Thomas, T, and Sung, R., *Plant Molecular Biology* 12: 475-486.

The foregoing represent just some of the loci that were known as of March 2, 1995; well prior to the filing of the instant application. More than 25 regulatory elements were also described therein, as were numerous transformation vectors comprising combinations of these elements. Appellants could describe many more examples of traits that were well known as of the filing date, and would be glad to do so should the Board find it useful. It thus goes without saying that numerous traits were more than well known to those of skill in the art as of the filing date and were fully described in the specification.

Techniques for the introduction of converted locus traits by genetic transformation were further well known to those of skill in the art. Some of the transformation methods for corn that were well known as of the filing date and cited in the specification include the following: electroporation (U.S. Patent No. 5,384,253), microprojectile bombardment (U.S. Patent No. 5,550,318; U.S. Patent No. 5,736,369, U.S. Patent No. 5,538,880; and PCT Publication WO 95/06128), *Agrobacterium*-mediated transformation (U.S. Patent No. 5,591,616 and E.P. Publication EP672752), direct DNA uptake transformation of protoplasts (Omirulleh *et al.*,

1993) and silicon carbide fiber-mediated transformation (U.S. Patent No. 5,302,532 and U.S. Patent No. 5,464,765). Introduction of such traits by conventional breeding was also known. In fact, this is one of the most fundamental procedures in agricultural science, and it has not been alleged that this has not been described.

Appellants have therefore shown possession of the claimed male sterile plants and locus conversions. Both large numbers of male sterility genes and converted locus traits and the associated phenotypes were well known to those of skill in the art. The specification itself defines a locus converted plant as comprising essentially all of the desired morphological and physiological characteristics of the starting non-converted plant, *e.g.*, I113752. Well more than an adequate number of examples have been provided and were known in the art to satisfy written description. The state of the art must be considered in the written description determination. As such, Appellants respectfully request reversal of the rejection.

b) F1 Hybrid plants have been fully described

(1) The claimed hybrid plants share the genetic complement of corn variety I113752

The Examiner rejected claims directed to F1 hybrid plants and seeds produced with corn plant I113752 as one parent. Appellants have fully described this claimed subject matter in compliance with the written description requirement of 35 U.S.C. §112, first paragraph. As set forth in the breeding history in the specification, corn plant I113752 is an inbred corn plant. All of the claimed hybrid plants having I113752 as a parent will therefore contain a copy of the same genome as corn plant I113752. That is, because I113752 is an inbred corn plant, hybrid corn plants derived therefrom will have as half of their genetic material the same genetic contribution of corn plant I113752, save the possibility of the rare spontaneous mutation or undetected segregating locus. This entire genetic contribution of corn plant I113752 is described in the

specification by way of the proffered deposit of seed of corn plant I113752 with the ATCC. *See Enzo Biochem, Inc. v. Gen-Probe Inc.*, 296 F.3d 1316, 1330 (Fed. Cir. 2002) (holding that a biological deposit constitutes a written description of the deposited material under 35 U.S.C. §112, first paragraph). This represents a description of concrete and identifiable structural characteristics defining the claimed hybrid plants and distinguishing them from other plants in full compliance with the written description requirement.

The Federal Circuit has noted that such shared identifiable structural features are important to the written description requirement. *The Regents of The University of California v. Eli Lilly and Co.*, 119 F.3d 1559, 1568; 43 USPQ2d 1398, 1406 (Fed. Cir. 1997) (noting that a name alone does not satisfy the written description requirement where "it does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, *as one can do with a fully described genus, visualize or recognize the identity of the members of the genus*" (emphasis added)). Here, all of the members of the claimed genus of hybrids having I113752 as one parent share the structural feature of having the genetic complement of I113752. One of skill in the art could thus readily identify the members of the genus. The written description requirement has, therefore, been fully complied with.

(2) The shared characteristics of the claimed hybrid plants are readily identified and described in the specification

As set forth above, the claimed F1 hybrid plants having I113752 as one parent will share the same genetic complement received from I113752. This is readily identifiable by genetic marker analysis, as described in the specification. The same will be true for any hybrid plant having I113752 as one parent, save for an occasional difference at a locus due to spontaneous

genetic rearrangements, which occur at statistically insignificant frequencies in essentially all organisms.

The second plant that is used to make the claimed hybrid plants is irrelevant, as a hybrid will be produced any time corn plant I113752 is crossed with a second plant. That is, any second plant capable of reproduction may be used to make the hybrid plant. Appellants cannot therefore be said to lack written description for the second genetic complement. This is particularly so given that hundreds or even thousands of different inbred corn lines were well known to those of skill in the art prior to the filing of the instant application, each of which could be crossed to make a hybrid plant within the scope of the claims. This is evidenced by a review of the U.S.P.T.O. patent data website, which reveals more than 300 utility patents issued on different corn varieties issued prior to the filing date of the current application. Any one of these corn plants, or the many hundreds or thousands of other maize plants that were known at the time the application was filed, could be used to produce an F1 hybrid plant having corn variety I113752 as one parent, and each of these would share the genetic complement of I113752.

Written description is reviewed from the perspective of one of skill in the art at the time the application is filed. *Wang Labs., Inc. v. Toshiba Corp.*, 993 F.2d 858, 863 (Fed. Cir. 1993). The specification need not disclose what is well-known to those skilled in the art and preferably omits what is well-known and already available to the public. *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991). As ***any*** second plant may be used to produce the claimed hybrid plants and such plants were well known to those of skill in the art, Appellants cannot be said to have not been in possession of the second parent plant. The claimed hybrid corn plants have therefore been described in compliance with 35 U.S.C. §112, first paragraph.

(3) The entire genetic complement of corn variety I113752 is described by way of the proffered deposit of seed

The Federal Circuit has recently held that a biological deposit may be used to satisfy written description for nucleic acids, whether the nucleic acid sequence is set forth in the specification or not. Specifically, in *Enzo Biochem, Inc. v. Gen-Probe Inc.*, the patent owner had deposited six strains of *N. gonorrhoeae* and claimed nucleotide sequences hybridizing to the nucleic acids of these strains, but the patent application did not set forth the nucleic acid sequences of these strains in the specification. 296 F.3d 1316, 1328 (Fed. Cir. 2002). The Federal Circuit nonetheless held that "as those bacteria were deposited, their ***bacterial genome is accessible*** and, under our holding today, they are ***adequately described in the specification by their accession numbers***." *Id.* (emphasis added). In its holding, the Federal Circuit considered the burden that would be placed on applicants were they required to sequence each of the strains, noting lower court findings that it would have taken 3,000 scientists a month to sequence the bacterial genome of one strain of *N. gonorrhoeae*. *Id.* In the instant case, even more effort would be required, as corn is a higher life form with a more complex genome than the bacteria deposited in *Enzo*. The Examiner would nonetheless appear to require this much of Appellants in direct contradiction of *Enzo*.

The fact that the deposit here will be made after the filing date of the application has no bearing on written description, as the Federal Circuit has noted that insertion of an accession number for a deposit after the filing date adds no new matter to a case provided the deposited subject matter is clearly identified in the application. See *In re Lundak*, 773 F.2d 1216, 1217 (Fed. Cir. 1985) ("....an accession number and deposit date add nothing to the written description of the invention"). Appellants have therefore fully described the shared structure of

the claimed hybrid plants at the nucleic acid level and thus have fully complied with 35 U.S.C. §112, first paragraph.

(4) The Examiner's allegations that the expression of the genetic complement of corn variety I113752 is unpredictable are inapposite

The Examiner alleged that claimed hybrid plants have not been described despite inheriting the genetic complement of variety I113752 because information is not provided regarding the morphological and physiological traits of the hybrid plants. It is alleged that how the genes that are inherited would be expressed or would interact has not been shown. However, this misses the point that Appellants have gone one step further than morphological and physiological traits by describing the claimed hybrid plants at the genetic level. A better description could not be made than at the genetic level. Morphological and physiological traits, while helpful, are also subject to environmental variation and require subjective gradations. Genetic testing goes to the source of traits and yields concrete values.

The law further makes no distinctions regarding the manner in which applicants choose to describe claimed compositions. Rather, an applicant must merely describe the claimed subject matter by "whatever characteristics sufficiently distinguish it." *Amgen v. Chugai Pharmaceutical*, 927 F.2d 1200, 1206 (Fed. Cir. 1991). Here, Appellants have described the genetic complement of parent plant I113752 that will be comprised in the claimed hybrid plants. Indeed, Appellants describe the entire genetic complement of parent plant I113752 by way of a seed deposit made with the ATCC, as set forth above, as described above.

(5) Appellants describe exemplary hybrids made using inbred I113752

Further description of claimed hybrid plants is also provided in the specification by way of a detailed description of exemplary hybrids produced with I113752 as one inbred parent.

These plant are representative of hybrids produced using I113752 as one parent, each of which comprise the genetic complement of the parent corn plant as set forth above. The tables of the specification give the performance characteristics for the hybrids and provides comparisons against other hybrid varieties. In the tables, the morphological traits of the hybrid plants are given. This information, combined with the descriptions of I113752 in the specification and the shared structure among hybrids having corn plant I113752 as a parent, is more than adequate to describe the claimed subject matter.

c) Methods of Plant Breeding Are Fully Described

The Examiner rejected claims covering methods of plant breeding that comprise using variety I113752 as starting material as lacking written description. For example, it was apparently the position of the examiner that plants created at any intermediate or penultimate step are not described by specific structure yet must be under §112. However, what is required to meet the written description requirement is that an Applicant show that he or she was in possession of the ***claimed invention***. *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64 (Fed. Cir. 1991). Here, a process is claimed, not a product of a process, and thus the steps of that process must be described, not intermediate or final products of the steps. The starting materials for the process must also be provided, otherwise the process could not be completed. However, the only starting materials required are corn variety I113752, which the Examiner does not allege to have not been described, and ***any*** second corn plant. As set forth above, corn plants were well known, and this has also therefore been fully described.

With respect to the steps, these have been fully set forth in the claim. No essential steps have been shown to be absent. All that is required to complete the claimed method is to cross the corn variety I113752 or a product that is produced by any preceding step according to the steps given. All of the starting materials for any step within the method are either (1) corn variety

I113752, (2) any second corn plant, or (3) a corn plant that is produced by following a preceding method step. The method has therefore been fully described.

It is also noted that corn breeding is well known to those of skill in the art. Without it, there would not be commercial corn varieties, which are typically sold as hybrids produced by crossing two inbred varieties. This is evidenced by the more than 300 issued patents to inbred maize varieties discussed above, given that inbred plants are not produced without multiple generations of intentional self-fertilization breeding steps. All of the steps recited in the claim are typical of the process used for the production of new corn varieties, save for the point of novelty, corn variety I113752. This is evidenced in the breeding history for the production of corn variety I113752, which is given in the specification. The specification also describes methods for producing new corn varieties in the review of related art, for example, at pages 2-4 of the application.

In conclusion, all steps of the claimed process have been recited, all starting materials have been fully described, and methods of producing new corn varieties were well known to those of skill in the art. The claims are therefore been fully described in compliance with 35 U.S.C. §112, first paragraph.

In view of the foregoing, reversal of the rejection is thus respectfully requested.

D. The Claims Are Enabled Under 35 U.S.C. §112, First Paragraph

The Examiner rejected claims 6, 11 and 15-24 as not enabled. The claims are directed to corn plants of the claimed variety which comprise a locus conversion or a nuclear or cytoplasmic gene conferring male sterility as well as methods of plant breeding. The Examiner alleged that no guidance has been provided for creation of such plants and asserted non-enablement because absolute purity of backcrossed progeny may not be retained. However, this ignores the working

example in the specification describing an exemplary locus conversion that was made with a proprietary corn variety. This example gives the breeding history of the conversion that was made, including a description of seven backcrosses. The example describes exactly the type of process one of skill in the art could use to prepare conversions of the instant variety. The specification provides in great detail further guidance for creation of converted plants at pages 29-33. The techniques recited are also all well known in the art (e.g., Poehlman et al., 1995; Fehr, 1987; Sprague and Dudley, 1988).

With regard to creation of male sterile plants, this is also a technique that has been well-known for decades. This is evidenced by the numerous issued patents for creation of male sterile plants (see U.S. Patent No. 3,861,709; U.S. Patent No. 3,710,511; U.S. Patent No. 4,654,465; U.S. Patent No 5,625,132; U.S. Patent No. 4,727,219; U.S. Patent No. 5,530,191; U.S. Patent No. 5,689,041; U.S. Patent No. 5,741,684; and U.S. Patent No. 5,684,242, incorporated by reference).

The basis alleged by the Examiner for the rejection is a citation to a number of references said to show the difficulty of breeding corn plants as well as making male sterile or converted plants. These references do not support the rejection and in fact affirmatively demonstrate the enablement of the claims. For example, the Examiner cited Murray *et al.* (pp72-87, *Proc. 43rd Annual Corn and Sorghum Industry research*, Wilkinson *et al. eds.* 1988 American Seed Trade Assn.) for the proposition that linkage drag is common in corn breeding, that the "equivalent" of 10 backcrosses resulted in retention of 10% of the "unwanted" donor parent genome, this material would not be lost without backcrossing and selection, and that molecular marker assisted breeding or pedigree determination in corn is unpredictable due to a failure to identify markers specific for particular cultivars. Appellants traverse as the statements made regarding

this ***1988*** reference both selectively misquote the teachings while ignoring directly contradictory statements and have no negative bearing on enablement.

The sections cited for the proposition that linkage drag is common and regarding the "equivalent" of 10 backcrosses are misleading, for example, because in fact the authors indicate that only six backcrosses were carried out yet the authors were able to obtain plants that "approximated BC 10 in terms of inbreeding equivalence." Murray at p. 82, 2nd full ¶. There is further no basis to conclude why any additional number of generations of backcrossing would require undue experimentation. The example in the specification mentioned above shows multiple generations and any additional number of generations could be carried out using the most basic of experimentation.

Murray was also published in ***1988***, which Appellants submit is in no way indicative of the state of the art as of the filing date of the current application. As of March, 2002 the public USDA linkage map for maize included at least 1,006 RFLP and 804 SSR loci. In contrast, Murray states that "the current Agrigenetics maize RFLP linkage map consists of 300 independent loci." *See* p. 74, legend of Fig. 2; *see also* http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=136896.

Despite the relatively unadvanced state of the art at the time Murray *et al.* was published, the reference in fact affirmatively establishes enablement. Murray *et al.* note, for example, that:

> ***Polymorphism is relatively easy to detect in maize*** for several reason. First, maize exhibits considerable genetic variability. Second transposition, a predominant feature of maize genome evolution, generates easily detectable polymorphism []. However, transposition is not frequent enough to cause problems in the stability and utility of most probes []. Thus, in contrast to other crops such as tomato, soybean and lettuce [], it is possible to construct maize genetic maps using only a few different restriction enzymes.

Murray at p. 76, 2nd full ¶, (citations omitted; emphasis added). Still further, the reference notes the following:

> Heretofore, the process of genome recovery could only be estimated by phenotype, statistical expectation, and expensive combining ability tests. RFLP technology, on the other hand, ***reveals the mosaic of parental chromosome segments*** within each individual, thus ***permitting the breeder to select with accuracy never before possible.***

Murray at p. 82, 1st full ¶ (emphasis added).

Finally, Murray states in another section flanking a portion cited by the Examiner as showing problems associated with linkage drag that:

> The longstanding concept of using markers flanking a desirable gene to circumvent these problems is now practical with RFLP markers. ***Individuals in which recombination has occurred optimally close to the desired locus can be identified and thus linkage drag can be greatly reduced.*** This technique would involve searching among a progeny with the RFLP assay to discover individuals in which the desired recombination has occurred, and then using these individual for the next backcross []. In addition, any ***extraneous segments could be easily identified*** or at least tested for their contribution to phenotype.

Murray at p. 84, 2nd full ¶ (citation omitted; emphasis added). This section in and of itself establishes that all of the other assertions regarding an inability to introgress a locus conversion are completely unfounded and that those of skill in the art knew how to introgress such a conversion at least as of the ***1988*** publication of this paper, even using the relatively lightly populated RFLP map that existed at the time. All that is required to eliminate linkage drag is to select those individuals with recombinant segments. As stated by Murray, even as of the 1988 publication date markers could readily be used to carry this out. As of the filing date of this application, extensive public marker maps existed with at least 1,006 RFLP markers in addition to 804 SSR markers, which are both highly informative and amenable to high-throughput screening via PCR. Enablement is thus demonstrated by this reference.

With regard to the Kevern (US 5,850,009) and Carlone (US 5,763,755) references, these are irrelevant to enablement because the teachings cited do not even relate to backcrossing or introgression of a locus conversion. See col. 4, l. 37-46 of Kevern and paragraphs bridging cols.

1-2 of Carlone. Neither reference states that backcrossing or introgression of traits is unpredictable. In fact, Carlone states that "backcrossing for example, can be used to improve an inbred line" and that "[b]ackcrossing can be used to transfer a specific desirable trait from one inbred or source to an inbred that lacks that trait." Col. 3, l. 4-7. The reference even teaches a method for accomplishing this and states that selfing in the last backcross generation gives pure breeding progeny for the gene(s) being transferred. Col. 3, lines 4-21. These references contain no information suggesting non-enablement of the claims and again demonstrate that backcrossing was routine in the art.

The Examiner next cites Goldman *et al.* (*Crop Sci.*, 34:908-915, 1994) for the proposition that use of molecular markers to facilitate identification of chromosomal regions associated with quantitatively inherited traits is hampered by different linkage groups with different parents or that quantitative traits such as oil or protein content are inversely proportional to kernel size. In response, Appellants note again that the teaching and relevance of the reference have been misstated. This ***1994*** reference did not concern introgression of a trait into a starting genotype but rather involved mapping of complex QTLs in a population derived from the same starting line, the open pollinated variety Burr's White. See p. 909, col. 1, 1st ¶. Given the common ancestry it is not surprising that some markers were shared. Despite this, the conclusions drawn from the results of the studies is that "Results from this investigation demonstrate the effectiveness of identification of QTL for oil concentration and kernel weight in a population descended from a cross of two strains divergently selected for protein concentration. Quantitative trait loci for oil were detected despite a relatively narrow range of oil concentration." See. "Conclusions" starting at p. 913, col. 2, last ¶. Therefore, if any

conclusions can be drawn they are in favor of the ability to use marker assisted selection rather than against.

Next, Stuber (*Crop Sci.* 17(4):503-506, July, 1977), is cited for the proposition that corn breeding is confounded by epistasis and that grain and ear number were strongly affected by environment. In response, Appellants note that this ***1977*** reference was published so long ago as to be completely useless in drawing any inferences about what the state of the art was as of the filing date. Further, the reference relates to hybrid plant performance. See Abstract at p. 503. The reference is therefore irrelevant to the claims.

Finally, Melchinger (*Theor. Appl. Genet.*, 72:231-239, 1986) is cited for the proposition that epistasis reduced the amount of heterosis (hybrid vigor) in hybrid crosses. In response, Appellants note that, in addition to being published so long ago as to be useless, the reference is completely irrelevant. The claims do not require any level of heterosis nor is any such requirement made by any patent law. All that is required for enablement is objective enablement, not any particular level of efficacy. *In re Marzocchi*, 169 UPSQ 370 (CCPA 1971). Thus, even if the assertions made were taken as true, which is not conceded, this has no relevance to the claims or enablement.

In sum, no basis for doubting the enablement of the claims has been provided. Corn breeding is extremely advanced and well known in the art. This is due in large part to the fact that corn is one of the world's major food crops and largest seed crops. As explained in the specification, North American farmers alone plant tens of millions of acres of corn at the present time and there are extensive national and international commercial corn breeding programs. Appellants have more than adequately demonstrated that at most routine experimentation using

well know techniques would be required to practice the full scope of the claims. Reversal of the rejection is thus respectfully requested.

E. The Claims Are Not Anticipated Under 35 U.S.C. §102

The Examiner rejected claim 11 under 35 U.S.C. §102 as being anticipated by a patent said to teach some of the traits of the claimed variety. Appellants respectfully traverse as no showing that the reference actually does teach the claim limitations or for concluding that such properties are inherent has been made on the record. For example, claim 11 is directed to a corn plant that is capable of expressing all of the physiological and morphological characteristics of the corn variety I113752. Absolutely no basis for concluding that any prior art plant is capable of expressing these characteristics has been provided and the characteristics of variety I113752 are already admittedly novel. Hypothetical possibilities in a rejection do not suffice.

The Examiner made no attempt to show that the cited variety actually does anticipates the subject matter of the claims. Under 35 U.S.C. § 102(b) it is the burden of the Office to show that each and every element as set forth in the claim is found in the prior art. *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). The identical invention must be shown in as complete detail as is contained in the claim. *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989). This has not been done and the anticipation rejection must therefore fail.

To the extent that unexpressed inherent characteristics of the cited variety form the basis of the anticipation rejection, it is noted by Appellants that these characteristics must necessarily flow from the prior art. *Continental Can Co. USA v. Monsanto Co.*, 948 F.2d 1264, 1268, 20 USPQ2d 1746, 1749 (Fed. Cir. 1991) ("To serve as an anticipation when the reference is silent about the asserted inherent characteristic, such gap in the reference may be filled with recourse to

extrinsic evidence. Such evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill."). Here, it is merely stated that the cited variety shares some of the same characteristics and there is no showing that any other traits are necessarily present. The Examiner has thus failed to meet the burden under 35 U.S.C. §102.

In view of the foregoing, reversal of the rejection is respectfully requested.

CONCLUSION

It is respectfully submitted, in light of the above, that none of the claims are properly rejected. Therefore, Appellants request that the Board reverse the pending grounds for rejection.

Respectfully submitted,

Robert E. Hanson
Reg. No. 42,628
Attorney for Appellants

FULBRIGHT & JAWORSKI L.L.P
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 536-3085

Date: May 30, 2006

VIII. CLAIMS APPENDIX

6. (Original) The corn plant of claim 2, further comprising a nuclear or cytoplasmic gene conferring male sterility.

11. (Original) A corn plant regenerated from the tissue culture of claim 9, wherein the corn plant is capable of expressing all of the physiological and morphological characteristics of corn variety I113752, wherein a sample of the seed of the corn variety I113752 was deposited under ATCC Accession No. - - - - -.

15. (Original) The corn plant of claim 2, further comprising a transgene introduced by genetic transformation.

16. (Original) The corn plant of claim 15, wherein the transgene confers a trait selected from the group consisting of herbicide tolerance, insect resistance, disease resistance, yield enhancement, waxy starch, improved nutritional quality, decreased phytate content, modified fatty acid metabolism, modified carbohydrate metabolism, male sterility and restoration of male fertility.

17. (Original) A method of producing a transgenic corn plant, comprising introducing a transgene into a plant of corn variety I113752, wherein a sample of the seed of the corn variety I113752 was deposited under ATCC Accession No. - - - - -.

18. (Original) A method of producing an inbred corn plant derived from the corn variety I113752, the method comprising the steps of:

(a) preparing a progeny plant derived from corn variety I113752 by crossing a plant of the corn variety I113752 with a second corn plant, wherein a sample of the seed of the corn variety I113752 was deposited under ATCC Accession No. - - - - -;

(b) crossing the progeny plant with itself or a second plant to produce a seed of a progeny plant of a subsequent generation;

(c) growing a progeny plant of a subsequent generation from said seed and crossing the progeny plant of a subsequent generation with itself or a second plant; and

(d) repeating steps (b) and (c) for an additional 2-10 generations to produce an inbred corn plant derived from the corn variety I113752.

19. (Previously presented) A method of producing a conversion of the corn variety I113752 to express at least one new trait, the method comprising the steps of:

(a) crossing a first corn plant having a first diploid genome comprising a plurality of paired chromosomes comprising a plurality of mappable genetic loci with a pair of alleles at each locus, and further comprising a conversion that confers at least one new trait, with a second plant of the corn variety I113752, a sample of the seed of the corn variety I113752 having been deposited under ATCC Accession No. - - - - -, the plant of the corn variety I113752 having a second diploid genome comprising a plurality of paired chromosomes comprising a plurality of mappable genetic loci with a pair of alleles at each locus, to produce seed comprising a diploid genome having a plurality of paired chromosomes comprising a plurality of mappable genetic loci with a pair of alleles at each locus, wherein one of the alleles is contributed by the first corn plant and the other is contributed by the plant of the corn variety I113752, said genome further comprising the conversion that confers the new trait;

(b) harvesting and planting the seed thereby produced to produce at least one progeny plant of the first filial generation, said progeny plant comprising a diploid genome comprising the conversion;

(c) crossing said progeny plant with a plant of the corn variety I113752 to produce seed of a subsequent filial generation, the seed comprising a diploid genome having a plurality of paired chromosomes comprising a plurality of mappable genetic loci with a pair of alleles at each locus, wherein one of the alleles is contributed by the progeny plant and the other is contributed by the plant of the corn variety I113752, and further comprising the conversion that confers the new trait;

(d) growing at least one progeny plant of the subsequent filial generation from the seed produced in step (c), said progeny plant comprising a genome comprising the conversion that confers the new trait;

(e) repeating steps (c) and (d) for at least one additional generation to produce a converted plant of the corn variety I113752 wherein the plant comprises a diploid genome having a plurality of paired chromosomes comprising a plurality of mappable genetic loci with a pair of alleles at each locus, wherein both alleles at substantially all of the loci consist essentially of the allele found at the same locus in corn variety I113752, the genome further comprising the conversion that confers the new trait; and

(f) harvesting the seed of the converted plant.

20. (Previously presented) The method of claim 19, wherein the conversion was stably inserted into a corn genome by genetic transformation.

21. (Previously presented) The method of claim 19, wherein the new trait is selected from the group consisting of herbicide tolerance; insect resistance; disease resistance; waxy starch; decreased phytate content, modified fatty acid metabolism, modified carbohydrate metabolism; male sterility and restoration of male fertility.

22. (Original) A converted plant of the corn variety I113752 produced by the method of claim 19.

23. (Original) A hybrid corn seed having a male parent and a female parent, wherein the male and female parents each comprise a diploid genome having a plurality of paired chromosomes comprising a plurality of mappable genetic loci with a pair of alleles at each locus; the hybrid corn seed also comprising a diploid genome having a plurality of paired chromosomes comprising a plurality of mappable genetic loci with a pair of alleles at each locus, one of the alleles being contributed by the male parent and the other being contributed by the female parent, wherein one of the parents is a plant of the corn variety I113752, a sample of the seed of said corn variety I113752 having been deposited under ATCC Accession No. - - - - -, and wherein the

other parent is a plant of a different variety; whereby one allele at each locus in the hybrid genome consists essentially of the allele found at the same locus in corn variety I113752, and further whereby the other allele in a plurality of such loci in the hybrid genome is different from the allele found at the same locus in corn variety I113752.

24. (Original) A corn plant grown from the seed of claim 23.

IX. EVIDENCE APPENDIX

No exhibits

X. RELATED PROCEEDINGS APPENDIX

Exhibit A:	Copy of Board Decision in Appeal No. 2004-1506 (Ser. No. 09/788,334)
Exhibit B:	Copy of Board Decision in Appeal No. 2004-1968 (Ser. No. 10/000,311)
Exhibit C:	Copy of Board Decision in Appeal No. 2004-1503 (Ser No. 09/606,808)
Exhibit D:	Copy of Board Decision in Appeal No. 2004-2317 (Ser. No. 09/771,938)
Exhibit E:	Copy of Board Decision in Appeal No. 2004-2343 (Ser. No. 09/772,520)
Exhibit F:	Copy of Board Decision in Appeal No. 2005-0396 (Ser. No. 10/077,589)

EXHIBIT A

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

RECEIVED
Date/Docketed: Status Check Decision on Appeal Rec'd; Response from Examiner? 6/30/05
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Initials:

Ex parte Thomas B. Carlson

Appeal No. 2004-1506[1]
Application No. 09/788,334

Heard: February 10, 2005[2]

MAILED
MAR 31 2005
U.S. PATENT AND TRADEMARK OFFICE
BOARD OF PATENT APPEALS
AND INTERFERENCES

Before SCHEINER, ADAMS and GREEN, Administrative Patent Judges.

ADAMS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on the appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 3, 6, 11, 14-20 and 24-31. The examiner has indicated that claims 1, 2, 5, 7-10, 12, 13 and 21-23 are allowable. Answer, page 2. According to the examiner (id.; Accord, Brief, page 6), "[c]laim 4 has been cancelled in the paper submitted by [a]ppellant on 30 June 2003." In addition, appellant confirmed during the February 10th oral hearing that it was his

[1] This appeal is substantially similar to Appeal No. 2004-1503, Application No. 09/606,808; Appeal No. 2004-1968, Application No. 10/00,0311; Appeal No. 2004-2317, Application No. 09/771,938; Appeal No. 2004-2343, Application No. 09/772,520; and Appeal No. 2005-0396, Application No. 10/077,589, which all share the same assignee, Monsanto Company, the parent of wholly-owned subsidiary DeKalb Genetics Corporation. Accordingly we have considered these appeals together.

[2] We note that examiner Ashwin Meta presented arguments at the oral hearing.

intent to cancel pending claim 26, accordingly we have not considered this claim in our deliberations.

Claims 3, 6, 15, 16, 17, 27, 28 and 30 are illustrative of the subject matter on appeal and are reproduced below. In addition, for convenience, we have reproduced allowable claims 2 and 5 below:

2. A population of seed of the corn variety I015011, wherein a sample of the seed of the corn variety I015011 was deposited under ATCC Accession No. PTA-3224.

3. The population of seed of claim 2, further defined as an essentially homogeneous population of seed.

5. A corn plant produced by growing a seed of the corn variety I015011, wherein a sample of the seed of the corn variety I015011 was deposited under ATCC Accession No. PTA-3224.

6. The corn plant of claim 5, having:
   (a) an SSR profile in accordance with the profile shown in Table 5; or
   (b) an isozyme typing profile in accordance with the profile shown in Table 6.

15. A corn plant capable of expressing all the physiological and morphological characteristics of the corn variety I015011, wherein a sample of the seed of the corn variety I015011 was deposited under ATCC Accession No. PTA-3224.

16. The corn plant of claim 15, further comprising a nuclear or cytoplasmic gene conferring male sterility.

17. A tissue culture of regenerable cells of a plant of corn variety I015011, wherein the tissue is capable of regenerating plants capable of expressing all the physiological and morphological characteristics of the corn variety I015011, wherein a sample of the seed of the corn variety I015011 was deposited under ATCC Accession No. PTA-3224.

27. The corn plant of claim 5, further defined as having a genome comprising a single locus conversion.

28. The corn plant of claim 27, wherein the single locus was stably inserted into a corn genome by transformation.

30. The corn plant of claim 27, wherein the locus confers a trait selected from the group consisting of herbicide tolerance; insect resistance; resistance to bacterial, fungal, nematode or viral disease; yield enhancement; waxy starch; improved nutritional quality; enhanced yield stability; male sterility and restoration of male fertility.

The references relied upon by the examiner are:

Hunsperger et al. (Hunsperger) 5,523,520 Jun. 4, 1996

Eshed et al. (Eshed), "Less-Than-Additive Epistatic Interactions of Quantitative Trait Loci in Tomato," Genetics, Vol. 143, pp. 1807-17 (1996)

Kraft et al. (Kraft), "Linkage Disequilibrium and Fingerprinting in Sugar Beet," Theoretical and Applied Genetics, Vol. 101, pp. 323-36 (2000)

GROUNDS OF REJECTION

Claim 3 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "an essentially homogeneous population of seed."

Claim 14 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "[a]n essentially homogeneous population of corn plants produced by growing the seed of the corn variety I015011."

Claims 6 and 11 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "in accordance with."

Claims 15, and 17-20[3] stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "capable of expressing."

[3] According to the examiner (Answer, pages 12 and 13), since claims 18 and 19 depend from claim 17 they are included in this rejection.

Claims 16 and 27-30[4] stand rejected under 35 U.S.C. § 112, second paragraph as failing to limit the scope of the claims from which they depend.

Claim 28 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of "the article 'a' in the recitation 'wherein the single locus was stably inserted into a corn genome.'"

Claim 30 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrases "yield enhancement," "improved nutritional quality," and "enhanced yield stability."

Claims 6, 11, 24, 25 and 27-31[5] stand rejected under the written description provision of 35 U.S.C. § 112, first paragraph.

Claims 27-30 stand rejected under the enablement provision of 35 U.S.C. § 112, first paragraph.

We reverse.

BACKGROUND

The present "invention relates to inbred corn seed and plants of the variety designated I015011, and derivatives and tissue cultures thereof." Specification, page 1. According to appellant (specification, page 27), "[a] description of the physiological and morphological characteristics of corn plant

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[4] According to the examiner (Answer, page 4), "[c]laims ... 27-30 ... stand rejected under 35 U.S.C. [§] 112, second paragrarph..." The examiner, however, provides no explanation as to why claim 29 is rejected. We can only assume that since claim 29, as well as claims 28 and 30, each depend from claim 27, they are rejected for the same reason as claim 27. Accordingly, we have included claims 28-30 with this ground of rejection.

[5] While the examiner included claim 26 in this rejection, for the reasons set forth above, we have not considered this claim in our deliberations.

I015011 is presented in Table 3" of the specification, pages 27-29. On this record the examiner has indicated that claims drawn to plants, plant parts, and seed of the corn variety designated I015011 are allowable. See e.g., claims 1, 2, 5, 7-10, 12 and 13, and Answer, page 2, wherein the examiner states "[c]laims 1, 2, 5, 7-10, 12 [and] 13 ... are allowed."

A second aspect of the present invention comprises hybrid plants and processes "for producing [first generation (F_1) hybrid[6]] corn seeds or plants, which ... generally comprise crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is a plant of the variety designated I015011." Specification, pages 7-9. On this record the examiner has indicated that claims drawn to a process of producing corn seed wherein the process comprises crossing a first parent corn plant with a second parent corn plant are allowable. See e.g., claims 21-23 and Answer, page 2, wherein the examiner states claims "21-23 are allowed."

A third aspect of the present invention comprises single locus converted plants of the corn variety I015011. Specification, page 6. As appellant explains (specification, page 23, emphasis added), single locus converted (conversion) plants are those plants

> which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. A single

[6] According to the specification (page 21), a F_1 hybrid is "[t]he first generation progeny of the cross of two plants." During oral hearing, appellant confirmed that all claims drawn to hybrid plants or hybrid seeds (see e.g., claims 24 and 25) refer to F_1 hybrids.

> locus may comprise one gene, or in the case of transgenic plants, one or more transgenes integrated into the host genome at a single site (locus).

As appellant explains (specification, page 31):

> Many single locus traits have been identified that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques. Single locus traits may or may not be transgenic; examples of these traits include, but are not limited to, male sterility, waxy starch, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, male fertility, enhanced nutritional quality, industrial usage, yield stability, and yield enhancement. These genes are generally inherited through the nucleus, but may be inherited through the cytoplasm. Some known exceptions to this are genes for male sterility, some of which are inherited cytoplasmically, but still act as single locus traits.

A final aspect of the present invention is directed to a process of producing an inbred corn plant <u>derived</u> from a plant of the corn variety I015011. <u>See</u> <u>e.g.</u>, claim 31. According to appellant's specification (page 10),

> the present invention provides a method of producing an inbred corn plant derived from the corn variety I015011, the method comprising the steps of: (a) preparing a progeny plant derived from corn variety I015011, wherein said preparing comprises crossing a plant of the corn variety I015011 with a second corn plant, and wherein a sample of the seed of corn variety I015011 has been deposited under ATCC Accession No. ... [PTA-3224]; (b) crossing the progeny plant with itself or a second plant to produce a seed of a progeny plant of a subsequent generation; (c) growing a progeny plant of a subsequent generation from said seed of a progeny plant of a subsequent generation and crossing the progeny plant of a subsequent generation with itself or a second plant; and (d) repeating steps (c) and (d) for an addition 3-10 generations to produce an inbred corn plant derived from the corn variety I015011. In the method, it may be desirable to select particular plants resulting from step (c) for continued crossing according to steps (b) and (c). By selecting plants having one or more desirable traits, an inbred corn plant derived from the corn variety I015011 is obtained which possesses some of the desirable traits of corn variety I015011 as well potentially other selected traits.

According to the examiner (Answer, page 36), "[t]he patentability of the method of claim 31 does not lie in the method steps, which require the simple acts of crossing corn plants, allowing progeny seed to be produced, and growing progeny plants from the seed...." Therefore, as we understand this aspect of the claimed invention (e.g., claim 31), the intent is not to claim a specific inbred corn plant resulting from the claimed process. See claim 31. Instead, as we understand it, claim 31 is drawn to a process wherein an inbred corn plant is derived from the corn variety I015011.

As appellant explains (specification, page 3),

> The development of uniform corn plant hybrids requires the development of homozygous inbred plants, the crossing of these inbred plants, and the evaluation of the crosses. Pedigree breeding and recurrent selection are examples of breeding methods used to develop inbred plants from breeding populations. Those breeding methods combine the genetic backgrounds from two or more inbred plants or various other broad-based sources into breeding pools from which new inbred plants are developed by selfing and selection of desired phenotypes. The new inbreds are crossed with other inbred plants and the hybrids from these crosses are evaluated to determine which of those have commercial potential.

We emphasize, that while "new inbreds" having commercial potential may result from the method set forth in claim 31, the claim does not encompass any specific plant that is produced as a result of the method. Rather the claim encompasses only a method of producing an inbred corn plant that is "derived" from the corn variety I015011. The examiner has indicated that a claim drawn to a corn plant of the corn variety I015011 is allowable. See e.g., claim 5, and Answer, page 2, wherein the examiner states that claim 5 is allowed.

Against this backdrop, we now consider the rejections of record.

DISCUSSION

Definiteness:

Claims 3, 6, 11, 14-20 and 27-30 stand rejected under 35 U.S.C. § 112, second paragraph. For the following reasons we reverse.

Claim 3

Claim 3 depends from independent claim 2, and stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "an essentially homogeneous population of seed...." Answer, page 5. According to the examiner (id.), claim 2 is drawn to "'[a] population of seed of the corn variety I015011, wherein a sample of the seed of the corn variety I015011 was deposited under ATCC Accession No. PTA-3224." Thus, the examiner finds (id.), the population of seed set forth in claim 2 "is a homogeneous population of seed of corn variety I015011." Accordingly, the examiner finds (id.), "[t]he recitation, 'essentially homogeneous,' in claim 3 ... appear[s] to be superfluous."

However, as disclosed in appellant's specification (page 5),

> [e]ssentially homogeneous populations of inbred seed are those that consist essentially of the particular inbred seed, and are generally free from substantial numbers of other seed, so that the inbred seed forms between about 90% and about 100% of the total seed, and preferably, between about 95% and about 100% of the total seed.

Accordingly, we disagree with the examiner's assertion (Answer, page 6) that claim 3 is unclear simply because it may contain seed other than the seed of the corn variety I015011. We remind the examiner that claim language must be analyzed "not in a vacuum, but always in light of the teachings of the prior art

and of the particular application disclosure as it would be interpreted by one possessing the ordinary skill in the pertinent art." In re Moore, 439 F.2d 1232, 1235, 169 USPQ 236, 238 (CCPA 1971). Here, notwithstanding appellant's comments[7], it is our opinion that a person of ordinary skill in the art would recognize that an essentially homogeneous population of seed of the corn variety I015011 is a population of seed that is generally free from substantial numbers of other seed, e.g., wherein corn variety I015011 seed forms between about 90% and about 100% of the total seed in the population.[8]

Accordingly, we reverse the rejection of claim 3 under 35 U.S.C. § 112, second paragraph.

Claim 14

Claim 14 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "[a]n essentially homogeneous population of corn plants produced by growing the seed of the corn variety I015011." Answer, page 7. According to the examiner (Answer, page 7), "[t]he I015011 seed can only produce I015011 plants. ... [Therefore,] [t]he population can ... only consist of I015011 plants." Accordingly, the examiner finds it unclear "why the population is referred to as 'essentially homogeneous,' since such populations can comprise more than one variety of plant." Id.

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[7] According to appellant (Brief, page 7), an essentially homogeneous population of seed, is a population of seed that could be of non-uniform size and shape.

[8] Cf. the examiner's statement (Answer, page 6), "amending claim 3 to read '[a]n essentially homogeneous population of corn seeds consisting essentially of seed of claim 1', would obviate this rejection."

As appellant discloses (specification, page 6), "[t]he population of inbred corn seed of the invention can further be particularly defined as being essentially free from hybrid seed. The inbred seed population may be separately grown to provide an essentially homogeneous population of inbred corn plants designated I015011." As we understand the claim, growing the seed of claim 3, for example, would produce an essentially homogeneous population of corn plants of the corn variety I015011.[9]

In addition, we direct the examiner's attention to Appeal No. 2005-0396, wherein a claim similar to claim 14 was presented for our review. In Appeal No. 2005-0396, the examiner of record indicated that claim 14, directed to "[a]n essentially homogeneous population of corn plants produced by growing the seed of the corn variety I180580...." was allowable. Accordingly, we find that the examiner has treated claim 14 in a manner that is inconsistent with the prosecution of claim 14 in 2005-0396. As we understand it, the only difference between claim 14 as it appears in Appeal No. 2005-0396 and the instant appeal is the variety of corn seed from which the plant is produced.

Accordingly we reverse the rejection of claim 14 under 35 U.S.C. § 112, second paragraph.

[9] Cf. The examiner's statement (Answer, page 7), amending claim 14 "to read, '[a]n essentially homogeneous population of corn plants produced by growing a population of corn seed consisting essentially of the seed of corn plant I015011...' would obviate the rejection."

Claims 6 and 11

Claims 6 and 11 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "in accordance with." According to the examiner (Answer, page 9), it is unclear if a plant "that generally follows the trend of the profile of Table 5, but which differs at one or a few loci, [would] be considered in 'conformity' or 'in accordance' with the profile of Table 5."

On this record, we understand the phrase "in accordance with" as it is used in claims 6 and 11 to mean "the same"[10]. Stated differently, we understand the claims to read:

6. The corn plant of claim 5, having:
   (a) the same SSR profile as shown in Table 5; or
   (b) the same isozyme typing profile as shown in Table 6.

11. The plant part of claim 10, wherein said cell is further defined as having:
   (a) The same SSR profile as shown in Table 5; or
   (b) The same isozyme typing as shown in Table 6.

Accordingly we reverse the rejection of claims 6 and 11 under 35 U.S.C. § 112, second paragraph.

Claims 15 and 17-20

Claims 15, and 17-20 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "capable of expressing," or "capable of regenerating." According to the examiner (Answer, page 10), the claims do "not make clear if the plant actually expresses the traits, or when or

[10] During the February 10, 2005 oral hearing appellant's representative confirmed that the phrase "in accordance with" was intended to mean "the same."

under what conditions the traits are expressed." In this regard, the examiner finds (Answer, bridging paragraph, pages 10-11),

> while the plant has the capacity to express the characteristics, for some reason it may not. Certain characteristics of a plant are expressed only at certain times of its life cycle, and are incapable of being expressed at other times. The colors of flower parts such as silks, or fruit parts such as husks, are examples. The promoters of many genes conferring traits require a transcription factor to become active. Is a plant that has such a gene, but not the transcription factor, considered "capable of expressing" that gene, and the trait associated with that gene, and is such a plant encompassed by the claims?

To address the examiner's concerns, we find it sufficient to state that if a plant has the capacity to express the claimed characteristics it meets the requirement of the claim regarding "capable of," notwithstanding that due to a particular phase of the life cycle the plant is not currently expressing a particular characteristic. Alternatively, if a plant is incapable of expressing the claimed characteristics at any phase of the life cycle, because it lacks, for example, the "transcription factor" required for expression – such a plant would not meet the requirement of the claim regarding "capable of."

Here, we find the examiner's extremely technical criticism to be a departure from the legally correct standard of considering the claimed invention from the perspective of one possessing ordinary skill in the art.[11] In our opinion, a person of ordinary skill in the art would understand what is claimed. Amgen Inc. v. Chugai Pharmaceutical Co., Ltd., 927 F.2d 1200, 1217, 18 USPQ2d 1016,

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[11] Cf. Digital Equipment Corp. v. Diamond, 653 F.2d 701, 724, 210 USPQ 521, 546 (CA 1981).

1030 (Fed. Cir. 1991). We find the same to be true for the phrase "capable of" as set forth in claims 17-20.

Accordingly we reverse the rejection of claims 15, and 17-20 under 35 U.S.C. § 112, second paragraph.

Claims 16 and 27-30

Claims 16 and 27-30 stand rejected under 35 U.S.C. § 112, second paragraph as failing to limit the scope of the claims from which they depend. According to the examiner (Answer, page 11), since the plant set forth in claim 16 is male sterile it cannot express all the morphological and physiological characteristics of the male fertile corn variety I015011. Similarly, the examiner finds it unclear whether the plant set forth in claim 27 has all the characteristics of the plant set forth in claim 5, from which claim 27 depends. Id. In response, appellant asserts (Brief, pages 10-11), claims 16 and 27 simply add a further limitation to the claims from which they depend. We agree.

For example, claim 16 reads on a corn plant capable of expressing all the physiological and morphological characteristics of the corn variety I015011, further comprising a nuclear or cytoplasmic gene conferring male sterility. In our opinion, the claims reasonably apprise those of skill in the art of their scope. Amgen, As set forth in Shatterproof Glass Corp. v. Libbey-Owens Ford Co., 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir. 1985), "[i]f the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more."

Accordingly we reverse the rejection of claims 16 and 27-30 under 35 U.S.C. § 112, second paragraph.

Claim 28

Claim 28 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of "the article 'a' in the recitation 'wherein the single locus was stably inserted into a corn genome.'" According to the examiner (Answer, page 13), "[t]he recitation does not make clear if the genome is that of I015011 or that of a different corn plant."

According to appellant's specification (page 23, emphasis removed), a "Single Locus Converted (Conversion) Plant" refers to

> [p]lants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. A single locus may comprise one gene, or in the case of transgenic plants, one or more transgenes integrated into the host genome at a single site (locus).

Accordingly, we agree with appellant (Brief, page 12) "[t]he single locus referred to in claim 28 may or may not have been directly inserted into the genome of the claimed plant." As we understand the claim, and arguments of record, claim 28 presents two possibilities: (1) the single locus is directly inserted into the claimed plant and nothing further need be done; or (2) the single locus is directly inserted into a different plant, which is then used to transfer the single locus to the claimed plant through use of the plant breeding technique known as backcrossing.

In our opinion, the claim reasonably apprises those of skill in the art of its scope. Amgen. Accordingly, we reverse the rejection of claim 28 under 35 U.S.C. § 112, second paragraph.

Claim 30

Claim 30 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrases "yield enhancement," "improved nutritional quality," and "enhanced yield stability." According to the examiner the terms "yield enhancement," "improved nutritional quality," and "enhanced yield stability" are relative and have no definite meaning. Answer, page 14. The examiner is correct (Answer, page 14), when a word of degree is used appellant's specification must provide some standard for measuring that degree. Seattle Box. Co. v. Industrial Crating & Packing, Inc., 731 F.2d 818, 826, 221 USPQ 568, 573-574 (Fed. Cir. 1984).

On this record, appellant asserts (Brief, page 13), it is "understood the enhancement of yield or yield stability and improved nutritional quality is relative to a plant lacking the single locus. The metes and bounds of the claim are thus fully understood by one of skill in the art and the use of the terms is not indefinite." On reflection, we agree with appellant. The fact that some claim language is not mathematically precise does not per se render the claim indefinite. Seattle Box. As set forth in Shatterproof Glass, "[i]f the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more." In our opinion, a

person of ordinary skill in the art would have understood the enhancement of yield or yield stability and improved nutritional quality is relative to a plant lacking the single locus.

Accordingly we reverse the rejection of claim 30 under 35 U.S.C. § 112, second paragraph.

Written Description:

Claims 6, 11, 24, 25 and 27-31 stand rejected under 35 U.S.C. § 112, first paragraph, as the specification fails to adequately describe the claimed invention. For the following reasons, we reverse.

Claims 24 and 25[12]

Claims 24 and 25 both depend from claim 23. On this record, the examiner has indicated that claim 23 is allowable. Answer, page 2. The examiner finds (Answer, page 16), claims 24 and 25 are drawn to a hybrid plant or seed "produced by crossing inbred corn plant I015011 with any second, distinct inbred corn plant."

[12] We recognize, as does the examiner (Answer, page 21) that appellant's reference to claims 22-26 (Brief, page 14) was intended to be a reference to claims 24 and 25.

As we understand it, based on this construction of claims 24 and 25, the examiner is of the opinion that since the hybrids inherit only ½ of their diploid[13] set of chromosomes from the plant of corn variety I015011, a person of skill in the art would not have viewed the teachings of the specification as sufficient to demonstrate that appellant was in possession of the genus of hybrid seeds and plants encompassed by claims 24 and 25. According to the examiner (Answer, page 22), "[t]he fact that any hybrid plant will inherit half of its alleles from I015011 then does not provide sufficient description of the morphological and physiological characteristics expressed by the claimed hybrid plants."

There is no doubt that the expressed gene products of a hybrid plant, e.g., the morphological and physiological traits, of I015011 and a non-I015011 corn plant will depend on the combination of the genetic material inherited from both parents. See Answer, page 22. Nevertheless, we disagree with the examiner's conclusion (id.) that "[t]he fact that any hybrid plant will inherit half of its alleles from I015011 then does not provide sufficient description of the morphological and physiological characteristics expressed by the claimed hybrid plants."

On these facts, we find it necessary to take a step back and consider what is claimed. The claims are drawn to a F_1 hybrid seed (claim 24) or plant (claim 25) resulting from a cross between a plant of corn variety I015011 and a non-I015011 corn variety. The claims do not require the hybrid to express any particular morphological or physiological characteristic. Nor do the claims

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[13] According to appellant's specification (page 21), diploid means "a cell or organism having two sets of chromosomes."

require that a particular non-I015011 corn variety be used.[14] All that is required by the claims is that the hybrid has one parent that is a plant of corn variety I015011. Since the examiner has indicated that the seed and the plant of the corn variety I015011 are allowable (see claims 1 and 5), there can be no doubt that the specification provides an adequate written description of this corn variety. In addition, the examiner appears to recognize (Answer, pages 24-25) that appellant's specification describes an exemplary hybrid wherein one parent was a plant of the corn variety I015011, see e.g., specification, pages 53-57. Accordingly, it is unclear to this merits panel what additional description is necessary.

As set forth in Reiffin v. Microsoft Corp., 214 F.3d 1342, 1345, 54 USPQ2d 1915, 1917 (Fed. Cir. 2000), the purpose of the written description requirement is to "ensure that the scope of the right to exclude, as set forth in the claims does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification." Here the hybrid seed or plant has one parent that is a plant of the corn variety I015011. To that end, to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). For the foregoing

[14] According to appellant (Brief, page 16), "hundreds or even thousands of different inbred corn lines were well known to those of skill in the art prior to the filing [date] of the instant application, each of which could be crossed to make a hybrid plant with in the scope of the claims."

reasons it is our opinion that appellant has provided an adequate written description of the subject matter set forth in claims 24 and 25.

We recognize the examiner's argument relating to SSR and isozyme markers (Answer, pages 25-30), as well as the examiner's arguments concerning a correlation between the hybrid's genome structure and the function of the hybrid plant (Answer, pages 23-25). However, for the foregoing reasons, we are not persuaded by these arguments.

Claims 6 and 11

Claims 6 and 11 depend ultimately upon claim 5. On this record, the examiner has indicated that claim 5 is allowable. Answer, page 2.

According to the examiner (Answer, page 17), while the specification provides the locus names and allele numbers of the SSR markers, the specification does not provide the actual nucleotide sequences that make up the markers. According to the examiner (Answer, bridging paragraph, pages 17-18), "names of loci alone do not describe the structures of the markers themselves. Without a description of the sequences of the markers, one cannot confirm their presence." In response, appellant points out (Brief, page 19), "the SSR markers were from Celera AgGen, Inc., which provides a commercial service for genotyping of maize varieties." In other words, a person of ordinary skill in the art could use the commercially available service provided by Celera AgGen, Inc. to determine whether a corn plant produced by growing a seed of the corn variety I015011 has an SSR profile which is the same as that shown in Table 5. Therefore, it is unclear to this panel why the examiner believes that such a

disclosure fails to provide adequate written descriptive support for the claimed invention.[15] Accordingly, we are not persuaded by the examiner's argument.

Regarding the isozyme typing profile, the examiner notes (Answer, page 18), "9 of the 12 isozyme markers of I015011 in Table 6 are also found in at least two other corn varieties, those of the other plants of Table 6." Based on this observation, the examiner concludes (id., emphasis added), "the markers in Table 6 are not adequate to distinguish the claimed hybrids from other corn plants, as other corn plants contain almost all of the same markers.[16]" We find the examiner's logic somewhat inconsistent, the examiner recognizes that isozyme typing profiles of "other corn plants" are different, yet concludes that the different isozyme profiles are inadequate to distinguish the claimed hybrids from other corn plants. Accordingly, we are not persuaded by the examiner's argument.

The examiner finds (Answer, page 31), claims 6 and 11 require that the claimed plant or plant cell exhibit either the claimed SSR profile or the isozyme profile. According to the examiner (id.), "[t]he genome of the cells of the I015011 seed deposited with the ATCC has both the SSR profile and the isozyme typing profile shown in Tables 5 and 6 for that plant. No plant is described in the specification that has one genetic marker profile but not the other." The

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[15] We are not persuaded by the examiner's assertion (Answer, page 28) "that the [commercially available] service used to detect SSR markers is currently available is not a guarantee that it will remain so for the life of a patent issuing from the application." Cf. In re Metcalfe, 410 F.2d 1378, 1382, 161 USPQ 789, 792-3 (CCPA 1969).

[16] Stated differently, the examiner recognizes that the isozyme typing profiles of the corn plants are different.

examiner's concern appears to be misplaced. To the extent that the examiner is concerned that the claim is open to read on a plant other than a corn plant produced by growing a seed of the corn variety I015011, we remind the examiner that both claims 6 and 11 ultimately depend from claim 5[17], which is drawn to "[a] corn plant produced by growing a seed of the corn variety I015011...."

It appears that the examiner may have read claims 6 and 11 as drawn to a corn plant or plant cell having only one of the recited profiles. However, as we understand claims 6 and 11, determining whether the claimed corn plant (claim 6) or plant cell (claim 11) has one of the profiles does not mean that the plant, or plant cell would not also exhibit the other profile.

In addition, we direct the examiner's attention to claims 6 and 11 of Appeal No. 2005-0396. As we understand it, notwithstanding differences in the SSR and isozyme profiles, the disclosure in the specification as well as the language of the claims is substantially similar to that of the instant application. Nevertheless, the examiner in Appeal No. 2005-0396 apparently found that appellant's specification provided an adequate written description of the claimed invention as no rejection of claims 6 and 11 was made under the written description provision of 35 U.S.C. § 112, first paragraph in Appeal No. 2005-0396. Accordingly, we find that the examiner has treated claims 6 and 11 in a manner that is inconsistent with the prosecution of similar claims in related application 10/077,589, which is the subject matter of Appeal No. 2005-0396.

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[17] The examiner has indicated that claim 5 is allowable. Answer, page 2.

For the foregoing reasons, we are not persuaded by the examiner's arguments.

Claims 27-30

According to the examiner (Answer, page 18), "[c]laims 27-30 are drawn towards I015011 plants further comprising a single locus conversion, or wherein the single locus was stably inserted into a corn genome by transformation." The examiner finds, however, that "the specification does not describe identified or isolated single loci for all corn plant traits." Answer, page 19. More specifically, the examiner finds (id.), claims 27-30 "broadly encompass single loci that have not been discovered or isolated." To the extent that the examiner is asserting that appellant has not provided an enabling disclosure of single loci that have not been identified, we note that to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath.

Nevertheless, it may be that the examiner's concern (Answer, page 31), is that "single genes that alone govern 'yield enhancement' or 'enhanced yield stability' have not been discovered." In this regard, the examiner asserts (Answer, page 34), "the references cited in the specification do not describe isolated single genes or loci that confer yield enhancement or yield stability." Therefore, the examiner concludes (id.), "[a]ppellant cannot be in possession of plants further comprising single loci that have yet to be identified." The examiner, however, provides no evidence to support the assertion that a person

of ordinary skill in the art would not recognize that single loci for yield enhancement or yield stability are known in the art. In this regard, we note that appellant discloses (specification, page 31), "[m]any single locus traits have been identified ... examples of these traits include, but are not limited to, ... enhanced nutritional quality, industrial usage, yield stability, and yield enhancement." It appears that the examiner has overlooked appellant's assertion that single locus traits for yield stability and yield enhancement are well known in the art. To this end, we direct the examiner's attention to, for example, United States Patent No. 5,936,145 ('145)[18], issued August 10, 1999, which is prior to the filing date of the instant application. For clarity, we reproduce claims 8, 29 and 39 of the '145 patent below:

> 8. A corn plant having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.
>
> 29. The corn plant of claim 8, further comprising a single gene conversion.
>
> 39. The single gene conversion of the corn plant of claim 29, where the gene confers enhanced yield stability.

As we understand it, claim 39 of the '145 patent, is drawn to a corn plant which comprises a single gene conversion, wherein the gene confers enhanced yield stability. Thus, contrary to the examiner's assertion it appears, for example, that a single gene that confers enhanced yield stability was known in the art prior to the filing date of the instant application. We remind the examiner "a patent need

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[18] We note that the assignee of the '145 patent is DeKalb Genetics Corporation. The assignee of the present application is Monsanto Company, the parent of wholly-owned subsidiary DeKalb Genetics Corporation.

not teach, and preferably omits, what is well known in the art." Hybritech Incorporated v. Monoclonal Antibodies, Inc. 802 F.2d 1367, 1385, 231 USPQ 81, 94 (Fed. Cir. 1986).

We remind the examiner that the inquiry into whether the description requirement is met must be determined on a case-by-case basis and is a question of fact. In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). A description as filed is presumed to be adequate; unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. See e.g., In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. Accordingly, it is the examiner who has the initial burden of establishing by a preponderance of evidence that a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. Wertheim, 541 F.2d at 263, 191 USPQ at 97. On this record, the examiner provides no evidence to support the assertion that single loci that govern, for example, yield enhancement or enhanced yield stability are not described.

For the foregoing reasons, we are not persuaded by the examiner's arguments.

Claim 31

Claim 31 is drawn to a method of producing an inbred corn plant derived from the corn variety I015011. The claimed method begins by crossing a plant of the corn variety I015011 with any other corn plant. The method requires that the

progeny corn plant be crossed either to itself, or with any other corn plant, and that the progeny of this cross be further crossed to itself, or with another corn plant, and so on throughout several generations. As we understand it, claim 31, in its simplest form, is directed to a method of using a plant of the corn variety I015011 to produce an inbred corn plant.

Nevertheless, the examiner finds (Answer, page 20), "[a] review of the claim indicates that hybrid progeny of corn plant I015011 are required to perform further crosses, and that progeny of subsequent generations can be further outcrossed with different corn plants." Therefore, the examiner concludes (id.), "[t]he hybrid progeny of corn plant I015011, and progeny plants of subsequent generations, are essential to operate the claimed method." As we understand the examiner's argument, not only does appellant have to provide a written description of the starting corn plant (I015011), but appellant also must look into the future to determine every other potential corn plant that someone may wish to cross with the I015011 corn variety, and provide written descriptive support for not only every other corn plant that could be crossed with I015011, but also the resulting progeny of each cross.

As set forth in Reiffin, the purpose of the written description requirement is to "ensure that the scope of the right to exclude, as set forth in the claims does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification." Here the method of producing an inbred corn plant requires a plant of the corn variety I015011 be used as the starting material. To that end, to satisfy the written description requirement, the inventor

"must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath. The examiner has indicated that a claim to a plant of the corn variety I015011 is allowable, see e.g., appellant's claim 5. Therefore, in our opinion, there can be no doubt that appellant was in possession of a plant of the corn variety I015011, in addition to a method of using that plant to cross with any other corn plant to produce an inbred corn plant as set forth in appellant's claim 31.

In our opinion, it matters not what the other corn plants are, or what the progeny of a cross between corn variety I015011 and some other corn plant represents. As the examiner explains (Answer, page 20), patentability of the method of claim 31 "does not lie in the method steps, which require the simple acts of crossing corn plants, allowing progeny seed to be produced, and growing progeny plants from the seed...." In our opinion, patentability of the method of claim 31 does not lie in the various other or second corn plants either. In our opinion, patentiblity of the method of claim 31 lies in the use of the corn variety I015011. Accordingly, for the foregoing reasons, it is our opinion that appellant has "convey[ed] with reasonable clarity to those skilled in the art that, as of the filing date sought, [they were] in possession of the invention," Vas-Cath (emphasis omitted).

Summary

For the foregoing reasons, we reverse the rejection of claims 6, 11, 24, 25 and 27-31 under the written description provision of 35 U.S.C. § 112, first paragraph.

Enablement:

Claims 27-30 stand rejected under the enablement provision of 35 U.S.C. § 112, first paragraph. The examiner finds (Answer, page 39), claims 27-30 "are broadly drawn towards inbred corn plant I015011 further defined as having a genome comprising any single locus conversion, encoding any trait; or wherein the single locus was stably inserted into a corn genome by transformation." The examiner presents several lines of argument under this heading. We take each in turn.

I. Retaining all the morphological and physiological traits of I015011:

According to the examiner (Answer, page 40, emphasis added), "the specification does not teach any I015011 plants comprising a single locus conversion produced by backcrossing, wherein the resultant plant retains all of its morphological and physiological traits in addition to exhibiting the single trait conferred by the introduced single locus. With reference to Hunsperger, Kraft, and Eshed the examiner asserts (Answer, page 43), "[t]he rejection raises the issue of how linkage drag hampers the insertion of single genes alone into a plant by backcrossing, while recovering all of the original plant's genome."

We note, however, that claims 27-30 do not require that the single locus conversion plant retain all of the morphological and physiological traits of the parent plant in addition to exhibiting the single trait conferred by the introduction of the single loci. Nor do claims 27-30 require that the resultant plant retain all of the original plant's genome in addition to the single locus transferred into the inbred via the backcrossing technique. As appellant explains (specification, bridging paragraph, pages 29-30, emphasis added),

> [t]he term single locus converted plant as used herein refers to those corn plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single locus transferred into the inbred via the backcrossing technique.

See also appellant's definition of single locus converted (conversion) plant at page 23 of the specification. We find nothing in the appellant's specification to indicate that the single locus converted plant retains all of the morphological and physiological traits, or all of the genome, of the parent plant in addition to the single locus transferred via the backcrossing technique. Accordingly, we disagree with the examiner's construction of claims 27-30 as "directed to exactly plant I015011 further comprising the single locus," which appears to disregard appellant's definition of a single locus converted plant. See Answer, page 44, emphasis added.

The examiner appears to appreciate (Answer, page 44) that appellant's specification provides an example of a converted plant. See e.g., specification, pages 35-36. However, for the foregoing reasons, we are not persuaded by the

examiner's assertion (Answer, page 44) that the specification provides "no indication that all of the morphological and physiological traits of [this converted] ... corn plant were recovered, and that only one single locus was transferred from the donor plant." To the contrary, the examiner provides no evidence that the converted plant exemplified in appellant's specification did not retain essentially all of the desired morphological and physiological characteristics of the inbred in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique.

Further, we recognize appellant's argument (Brief, page 29) that the examiner failed to establish a nexus between Hunsperger's discussion of petunias; Kraft's discussion of sugar beets; and Eshed's discussion of tomatoes, and the subject matter of the instant application - corn. Absent evidence to the contrary, we agree with appellant (id.), "[t]he [examiner's] indication[19] that the references concerning petunias, sugar beets and tomatoes apply to corn is made without any support." That the examiner has failed to identify (Answer, page 43) an example "in the prior art of plants in which linkage drag does not occur," does not mean that linkage drag is expected to occur in corn breeding, which according to appellant (Reply Brief, page 10) "is extremely advanced and well known in the art...." In this regard, we agree with appellant (Brief, pages 29-30; Accord Reply Brief, pages 10-11), the examiner has improperly placed the burden on appellant to demonstrate that the examiner's unsupported assertion is

[19] See Answer page 43, wherein the examiner asserts "[l]inkage drag appears to be a phenomenon that occurs in all plant types."

not true. We remind the examiner, as set forth in In re Wright, 999 F.2d 1557, 1561-62, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993):

> When rejecting a claim under the enablement requirement of section 112, the PTO bears an initial burden of setting forth a reasonable explanation as to why it believes that the scope of protection provided by that claim is not adequately enabled by the description of the invention provided in the specification of the application; this includes, of course, providing sufficient reasons for doubting any assertions in the specification as to the scope of enablement.

II. What plant is transformed in claim 28?

We recognize the examiner's assertion (Answer, page 41) that while claim 28 requires that a single locus be stably inserted into a corn genome by transformation, the claim does not indicate whether (1) the I015011 plant was transformed with the single locus, or (2) some other corn plant was transformed with the single locus and then introduced into I015011 by crossing. However, as appellant points out (Brief, page 12), claim 28 "specifies that the single locus was stably inserted into a corn genome. Loci that are stably inserted into a corn genome are also stably inherited. Thus the single locus need not have been inserted into the genome of corn variety I015011." Accordingly, the I015011 plant may be transformed with the single locus, or another plant may be transformed with the single locus and then introduced into I015011 by crossing.

It may be that the examiner is concerned that by transforming a non-I015011 plant with a single locus and then introducing this locus into I015011 by crossing would result in a plant that does not retain all of the morphological and

physiological traits, or all of the genome, of the I015011 plant. For the foregoing reasons, however, this line of reasoning is not persuasive.

III. The single locus to be introduced:

The examiner finds (Answer, page 41), "the claims do not place any limit on the single locus to be introduced" into I015011 plants. The examiner recognizes, however, that "[t]he prior art shows that hundreds of nucleotide sequences encoding products that confer various types of plant traits have been isolated at the time the instant invention was filed." Id. In addition, the examiner recognizes (id.), "[o]ne skilled in the art can transform any of these isolated nucleotide sequences known in the prior art into a corn plant cell, and regenerate a transgenic plant from the transformed cell."

Nevertheless, the examiner finds (Answer, page 42), "[u]ndue experimentation would be required by one skilled in the art to isolate single loci that govern the traits encompassed by the claims." In this regard, the examiner asserts (Answer, page 45) that the claims broadly encompass corn plants comprising any type of single loci, including those that have not yet been identified or isolated. To the extent that the examiner is asserting that appellant has not provided an enabling disclosure of single loci that have not been identified, we note that enablement under 35 U.S.C. § 112, first paragraph is evaluated as of appellant's filing date. As set forth in Chiron Corp. v. Genentech Inc., 363 F.3d 1247, 1254, 70 USPQ2d 1321, 1325-26 (Fed. Cir. 2004), "a patent document cannot enable technology that arises after the date of application. The law does not expect an applicant to disclose knowledge invented or

developed after the filing date. Such disclosure would be impossible. See In re Hogan, 559 F.2d 595, 605-06 [194 USPQ 527] (CCPA 1977)."

The examiner's comment, however, may be directed to his assertion (Answer, page 41) that "isolated loci whose products confer yield enhancement or enhanced yield stability (recited in claim 30), are not known in the prior art." However, as discussed, supra, it appears that contrary to the examiner's assertion a single locus that confers the trait of, for example, yield enhancement was known in the art prior to the filing date of the instant invention. In addition, as discussed, supra, appellant's specification asserts that such traits were known in the art. See specification, page 31. Accordingly, as set forth in In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971), the burden is on

> the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure.

On this record, we find only the examiner's unsupported conclusions as to why the specification does not enable the claimed invention. We remind the examiner that nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples. Marzocchi, 439 F.2d at 223, 169 USPQ at 369. In the absence of an evidentiary basis to support the rejection, the examiner has not sustained his initial burden of establishing a prima facie case of non-enablement.

In this regard, we note that the burden of proof does not shift to appellant until the examiner first meets his burden. Marzocchi, 439 F.2d at 223-224, 169 USPQ at 369-370.

We also recognize the examiner's assertion (Answer, page 42) that claims 27-29 "encompass plants with single loci whose functions are unknown … [or where] the effects of expression of the single locus on the traits expressed by I015011 are unknown." While this may be true, the examiner has not provided any evidence to suggest that it would require undue experimentation to obtain a single locus converted plant wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. See specification, page 23.

While it is not expressly stated in the text of the examiner's rejection, it may be that the examiner is concerned that the claims include inoperative embodiments. If so, the examiner is directed to Atlas Powder Co. v. E.I. DuPont De Nemours & Co., 750 F.2d 1569, 1576-77, 224 USPQ 409, 414 (Fed. Cir. 1984):

> Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. "It is not a function of the claims to specifically exclude ... possible inoperative substances...." In re Dinh-Nguyen, 492 F.2d 856, 859-59, 181 USPQ 46, 48 (CCPA 1974)(emphasis omitted). Accord, In re Geerdes, 491 F.2d 1260, 1265, 180 USPQ 789, 793 (CCPA 1974); In re Anderson, 471 F.2d 1237, 1242, 176 USPQ 331, 334-35 (CCPA 1971). Of course, if the number of inoperative combinations becomes significant, and in effect forces one of ordinary skill in the art to experiment unduly in order to practice the claimed invention, the claims might indeed be

> invalid. *See* e.g., In re Cook, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971).

On this record, the examiner provides no evidence that the number of inoperative embodiments is so large that a person of ordinary skill in the art would have to experiment unduly to practice the claimed invention. To the contrary, the examiner recognizes (Answer, page 41) that "[t]he prior art shows that hundreds of nucleotide sequences encoding products that confer various types of plant traits have been isolated at the time the instant invention was filed"; and that "[o]ne skilled in the art can transform any of these isolated nucleotide sequences known in the prior art into a corn plant cell, and regenerate a transgenic plant from the transformed cell." Accordingly, we are not persuaded by the examiner's unsupported assertions.

For the foregoing reasons, we reverse the rejection of claims 27-30 under the enablement provision of 35 U.S.C. § 112, first paragraph.

SUMMARY

We reverse the rejection of claims 3, 6, 11, 14-20, and 27-30 under 35 U.S.C. § 112, second paragraph.

We reverse the rejection of claims 6, 11, 24, 25 and 27-31 under the written description provision of 35 U.S.C. § 112, first paragraph.

We reverse the rejection of claims 27-30 under the enablement provision of 35 U.S.C. § 112, first paragraph.

Given appellant's stated intent to cancel claim 26, we did not considered the merits of the rejection of claim 26 under the written description provision of 35 U.S.C. § 112, first paragraph.

REVERSED

Toni R. Scheiner
Administrative Patent Judge

Donald E. Adams
Administrative Patent Judge

Lora M. Green
Administrative Patent Judge

BOARD OF PATENT APPEALS AND INTERFERENCES

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AUSTIN, TX 78701

EXHIBIT B

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

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Attorney(s): DLP, REH
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U.S. PATENT AND TRADEMARK OFFICE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte William D. Griffith

Appeal No. 2004-1968[1]
Application No. 10/000,311

ON BRIEF

Before SCHEINER, ADAMS and GREEN, Administrative Patent Judges.

ADAMS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on the appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 6, 12-19, 21, 24, 26-28 and 30-31. The examiner has indicated that claims 1-5, 7 and 9-11 are allowable. Page 3, Final Rejection, mailed July 1, 2003. Claims 20, 22, 23, 25, 29 and 32 are cancelled. The only remaining pending claim is claim 8. While appellant recognizes (Brief,

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[1] This appeal is substantially similar to Appeal No. 2004-1503, Application No. 09/606,808; Appeal No. 2004-1506; Application No. 09/788,334; Appeal No. 2004-2317, Application No. 09/771,938; Appeal No. 2004-2343, Application No. 09/772,520; and Appeal No. 2005-0396, Application No. 10/077,589, which all share the same assignee, Monsanto Company, the parent of wholly-owned subsidiary DeKalb Genetics Corporation. Accordingly we have considered these appeals together.

page 2) that claim 8 was rejected in the Final Office Action[2], appellant does not include claim 8 as part of the subject matter of the instant appeal. Id., see also, appellant's statement of the Issues on Appeal (Brief, page 3), which does not include claim 8. In this regard, we note, appellant's statement (Brief, page 19), "[t]he rejection of claim 8 concerns a minor clerical error easily corrected by amendment and thus has not been appealed. The examiner also recognized (Answer, bridging sentence, pages 2-3), "the indefiniteness rejection of claim 8 is not being contested...." Since appellant has conceded to the examiner's rejection of claim 8 and has not placed claim 8 before us on appeal, we have not considered claim 8 in our deliberations.

Claims 6, 12, 17, 19, 26 and 30 are illustrative of the subject matter on appeal and are reproduced below. In addition, for convenience, we have reproduced allowable claims 1, 2, and 11 below:

1. Seed of corn inbred line designated LH321, representative seed of said line having been deposited under ATCC Accession No. _____.

2. A corn plant, or parts thereof, produced by growing the seed of claim 1.

6. The corn plant of claim 2, wherein said plant is further defined as comprising a gene conferring male sterility.

11. A method for producing a hybrid corn seed comprising crossing a first inbred parent corn plant with a second inbred parent corn plant and harvesting the resultant hybrid corn seed, wherein said first inbred parent corn plant or second said parent corn plant is the corn plant of claim 2.

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[2] According to the examiner (page 3, Final Rejection, mailed July 1, 2003), claim 8 remains "rejected under 35 U.S.C. [§] 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention, as stated on pages 10-11 of the last Office [A]ction." At page 10 of this "last" Office Action, mailed January 13, 2003, the examiner finds "[c]laim 8 is indefinite in its recitation in line 1 of 'the...protoplasts' which lacks antecedent basis in claim 6. Amendment of claim 8, line 1 to delete 'the' before 'cells' would obviate this rejection."

12. A hybrid corn seed produced by the method of claim 11.

17. A method for producing inbred LH321 seed, representative seed of which have been deposited under ATCC Accession No. _____, comprising:
    a) planting a collection of seed comprising seed of a hybrid, one of whose parents is inbred LH321, said collection also comprising seed of said inbred;
    b) growing plants from said collection of seed;
    c) identifying inbred parent plants;
    d) controlling pollination in a manner which preserves the homozygosity of said inbred parent plant; and
    e) harvesting the resultant seed.

19. A method for producing a LH321-derived corn plant, comprising:
    a) Crossing inbred corn line LH321, representative seed of said line having been deposited under ATCC [A]ccession [N]umber _____, with a second corn plant to yield progeny corn seed; and
    b) Growing said progeny corn seed, under plant growth conditions, to yield said LH321-derived corn plant.

26. The corn plant, or parts thereof, of claim 2, wherein the plant or parts thereof have been transformed so that its genetic material contains one or more transgenes operably linked to one or more regulatory elements.

30. A method for developing a corn plant in a corn plant breeding program using plant breeding techniques comprising employing a corn plant, or its parts, as a source of plant breeding material comprising: using the corn plant, or its parts, of claim 2 as a source of said breeding material.

The references relied upon by the examiner are:

Hunsperger et al. (Hunsperger)	5,523,520	Jun. 4, 1996

Eshed et al. (Eshed), "Less-Than-Additive Epistatic Interactions of Quantitative Trait Loci in Tomato," Genetics, Vol. 143, pp. 1807-17 (1996)

Kraft et al. (Kraft), "Linkage Disequilibrium and Fingerprinting in Sugar Beet," Theoretical and Applied Genetics, Vol. 101, pp. 323-36 (2000)

GROUNDS OF REJECTION

Claim 6 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "further defined as comprising a gene conferring male sterility."

Claims 26-28 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "transformed so that its genetic material contains one or more transgenes."

Claims 6, 12-19, 21, 24, 26-28, 30, and 31 stand rejected under the written description provision of 35 U.S.C. § 112, first paragraph.

Claims 6, 12-19, 21, 24, 26-28, 30 and 31 stand rejected under the enablement provision of 35 U.S.C. § 112, first paragraph.

We reverse.

BACKGROUND

According to paragraph 23 of appellant's specification, the present invention

> relates to the seeds of inbred corn line LH321, to the plants [and plant parts] of inbred corn line LH321 and to methods for producing a corn plant produced by crossing the inbred line LH321 with itself or another corn line, and to methods for producing a corn plant containing in its genetic material one or more transgenes and to the transgenic corn plants produced by that method.

Paragraphs 44-55 of appellant's specification disclose morphologic and "other" characteristics of the inbred corn line LH321. On this record the examiner has indicated that claims drawn to plants, plant parts, and seed of the corn variety designated LH321 are allowable. See e.g., claims 1-5, 7, and 10, and page 3 of

the Final Rejection, mailed July 1, 2003, wherein the examiner states "[c]laims 1-5, 7 and ... [10] are allowed."

A second aspect of the present invention comprises "hybrid corn seeds and plants produced by crossing the inbred line LH321 with another corn line." Specification, paragraph 23, see also claims 12-16. On this record the examiner has indicated that claims drawn to a process of producing corn seed wherein the process comprises crossing a first parent corn plant with a second parent corn plant are allowable. See e.g., claim 11, and Final Rejection, mailed July 1, 2003, wherein the examiner states claim 11 is allowed.

A third aspect of the present invention is a corn plant from the inbred corn line LH321 further comprising "a cytoplasmic factor that is capable of conferring male sterility" (specification, paragraph 24); or transformed so that its genetic material contains one or more transgenes operably linked to one or more regulatory elements" (see e.g., claims 26-28). As appellant explains (specification, paragraph 13), "[I]t should be understood that the inbred can, through routine manipulation of cytoplasmic or other factors, be produced in a male-sterile form." According to appellant (specification, paragraph 67)

> scientists in the filed of plant biology developed a strong interest in engineering the genome of plants to confer and express foreign genes, or additional , or modified versions of native, or endogenous, genes (perhaps driven by different promoters) in order to alter the traits of a plant in a specific manner. Such foreign additional and/or modified genes are referred to herein collectively as "transgenes". Over the last fifteen to twenty years several methods for producing transgenic plants have been developed, and the present invention, in particular embodiments, also relates to transformed versions of the claimed inbred line.

A final aspect of the present invention is directed to a process of producing an inbred corn plant derived from a plant of the inbred corn line LH321 (see e.g., claims 11, 19 and 30), as well as hybrid plants and seed resulting from such a process (see e.g., claims 12-16). As discussed, supra, the examiner has indicated that claim 11 was allowable. According to appellant's specification (paragraph 56),

> [t]his invention is also directed to methods for producing a corn plant by crossing a first parent corn plant with a second parent corn plant, wherein the first or second corn plant is the inbred corn plant from the line LH321. Further, both first and second parent corn plants may be from the inbred line LH321. Therefore, any methods using the inbred corn line LH321 are part of this invention: selfing, backcrosses, hybrid breeding, and crosses to populations. Any plants produced using inbred corn line LH321 as a parent are within the scope of this invention.

Against this backdrop, we now consider the rejections of record.

DISCUSSION

Definiteness:

Claims 6 and 26-28 stand rejected under 35 U.S.C. § 112, second paragraph. For the following reasons we reverse.

Claim 6

According to the examiner (Answer, page 3), "the recitation 'further defined as comprising a gene conferring male sterility' ... appears to broaden the scope of its parent claim, or to raise some doubt as to whether the corn plant of claim 6 must be male sterile." In this regard, the examiner finds (id.), "[t]he specification does not define plants expressing all the physiological and morphological characteristics of LH321 as being male sterile, or as comprising a

gene that confers male sterility; in fact, the plant of claim 2 (from which claim 6 depends) is male fertile."

Initially, we note that claim 6 does not require that the corn plant express all the physiological and morphological characteristics of LH321. To the contrary, this appears to be the subject matter of claim 5, which the examiner has indicated to be allowable. Page 3, Final Rejection, mailed July 1, 2003. As we understand claim it, claim 6 is drawn to a corn plant, or parts thereof, produced by growing the seed of claim 1, wherein the plant or plant parts further comprise a gene conferring male sterility. In our opinion, claim 6 further limits the subject matter of claim 2, by requiring the plant of claim 2 to further comprise a gene conferring male sterility. Accordingly, we disagree with the examiner that claim 6 fails to further limit the subject matter of claim 2, from which it depends.

In addition, we fail to understand the examiner's statement that "claim 6 does not incorporate all elements of the parent claim [(claim 2)]." As discussed above, claim 6 depends from claim 2, thus all the elements of claim 2 are present in claim 6. Claim 6, however, possesses an additional limitation not found in claim 2 – a gene conferring male sterility. Thus, the male fertile plant of claim 2, is now male sterile as a result of the additional limitation added in claim 6. The examiner provides no evidence that male fertile plants cannot be made male sterile. To the contrary, we recognize the examiner's suggestion that appellant add two new claims drawn to (1) "[a] method of producing a male sterile corn plant comprising transforming the plant of claim 2 with nucleic acid

molecule that confers male sterility; and (2) "[a] male sterile corn plant produced by the ..." suggested method claim above.

Notwithstanding the examiner's assertion to the contrary, in our opinion, a person of ordinary skill in the art would understand what is claimed. Amgen Inc. v. Chugai Pharmaceutical Co., Ltd., 927 F.2d 1200, 1217, 18 USPQ2d 1016, 1030 (Fed. Cir. 1991). Accordingly, we reverse the rejection of claim 6 under 35 U.S.C. § 112, second paragraph.

Claims 26-28

According to the examiner (Answer, page 4), the recitation in claim 26 that the claimed corn plant be "'transformed so that its genetic material contains one or more transgenes' ... appears to broaden the scope of claim 2, or raises some doubt as to whether the plant has all of the traits expressed by the plant of claim 2." According to the examiner (id.), "[s]ince claim 2 is drawn to a plant with defined characteristics and genotypes which exclude the presence of introduced transgenes, it is confusing to characterize these plants as comprising additional genes." In addition, the examiner finds (id.), "[d]ependent claims 27-28 fail to remedy the deficiency of claim 26.

As with the discussion of claim 6 above, claim 26 simply adds a further limitation to claim 2. Specifically, that the plant or plant parts of claim 2 "have been transformed so that its genetic material contains one or more transgenes operably linked to one or more regulatory elements." Accordingly, notwithstanding the examiner's assertion to the contrary, in our opinion, a person of ordinary skill in the art would understand what is claimed. Amgen Inc. v.

Chugai Pharmaceutical Co., Ltd., 927 F.2d 1200, 1217, 18 USPQ2d 1016, 1030 (Fed. Cir. 1991). Accordingly, we reverse the rejection of claims 26-28 under 35 U.S.C. § 112, second paragraph.

Written Description:

Claims 6, 12-19, 21, 24, 26-28, 30 and 31 stand rejected under the written description provision of 35 U.S.C. § 112, first paragraph.

Claims 12-16

According to the examiner (Answer, page 7),

> A review of the language of claims 12-16 indicates that the claims are drawn to a genus, i.e., any and all hybrid corn seeds, and the hybrid corn plants produced by growing said hybrid seeds, wherein the hybrid seeds are produced by crossing inbred corn plant LH321 with any second, distinct inbred corn plant. Variation is expected in the complete genomes and phenotypes of the different hybrid species of the genus, since each hybrid has one non-LH321 parent that is not shared with the other hybrids. Each of the hybrids would inherit a different set of alleles from the non-LH321 inbred parent. As a result, the complete genomic structure of each hybrid, and therefore the morphological and physiological characteristics expressed by each hybrid, would differ.

Accordingly the examiner finds (Answer, page 13),

> [g]iven the lack of written description in the specification regarding any of a multitude of non-LH321 parents to be used in a backcrossing breeding method or any other classical breeding method, one skilled in the art would not have recognized Appellant to have been in possession of the claimed hybrids or progeny plants as recited in claims ... 12-16.

As we understand it, the examiner's concern (see e.g., Answer, pages 15-16) is that since the hybrids inherit only ½ of their diploid[3] set of chromosomes from the plant of corn variety LH321, a person of skill in the art would not have viewed the teachings of the specification as sufficient to demonstrate that appellant was in possession of the genus of hybrid seeds and plants encompassed by claims 12-16. According to the examiner (Answer, page 16), "[t]hat all hybrids will inherit half of their alleles from LH321 does not provide any information concerning the morphological and physiological characteristics that will be expressed by the claimed hybrids."

There is no doubt that the expressed gene products of a hybrid plant, e.g., the morphological and physiological traits, of LH321 and a non-LH321 corn plant will depend on the combination of the genetic material inherited from both parents. See Answer, page 22. Nevertheless, we disagree with the examiner's conclusion (id.) "[t]hat all hybrids will inherit half of their alleles from LH321 does not provide any information concerning the morphological and physiological characteristics that will be expressed by the claimed hybrids."

On these facts, we find it necessary to take a step back and consider what is claimed. As we understand the them, the claims are drawn to a F_1[4] hybrid seed (claims 12, 14 and 15) or plant/plant part (claim 13, and 16) resulting from a

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[3] According to appellant's specification (page 21), "[i]n a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes." Stated differently, diploid means a cell or organism having two sets of chromosomes.

[4] According to appellant's specification (page 3), "[a] single-cross hybrid is produced when two inbred lines are crossed to produce the F_1 progeny."

cross between the inbred corn plant LH321 and a non-LH321 corn plant. The claims do not require the hybrid to express any particular morphological or physiological characteristic. Nor do the claims require that a particular non-LH321 corn variety be used.[5] All that is required by the claims is that the F_1 hybrid has one parent that is a plant of corn variety LH321. Since the examiner has indicated that the seed and the plant of the inbred line LH321 are allowable (see claims 1 and 2), there can be no doubt that the specification provides an adequate written description of this inbred corn line. In addition, the examiner recognizes (Answer, page 7) that appellant's specification describes four exemplary hybrids wherein one parent was a plant of the inbred corn line LH321, see e.g., specification, pages 31-33. Accordingly, it is unclear to this merits panel what additional description is necessary.

As set forth above, the purpose of the written description requirement is to "ensure that the scope of the right to exclude, as set forth in the claims does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification." Reiffin. Here the F_1 hybrid seed or plant has one parent that is a plant of the inbred line LH321. To that end, to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). For the foregoing

[5] According to appellant (Brief, page 16), "hundreds or even thousands of other maize plants ... were known at the time the application was filed...."

reasons it is our opinion that appellant has provided an adequate written description of the subject matter set forth in claims 12-16. Accordingly, we reverse the rejection of claims 12-16 under the written description provision of 35 U.S.C. § 112, first paragraph.

<u>Claims 17-19, 21, 24, 30 and 31</u>[6]

According to the examiner (Answer, page 12), "[c]laims 19, 21, 24, 30 and 31 read on processes involving the repeated outcrossing of the exemplified inbred to multitude of genetically unrelated and uncharacterized corn plants for multiple generations." As the examiner explains (<u>id.</u>), "LH321 may be used only in the initial cross, and then the progeny of this cross may be crossed to a multitude of unrelated and uncharacterized corn plants for up to 7 times (as recited in claim 21) or <u>ad infinitum</u> (as claimed in claims 19, 24, 30 and 31)." In this regard, the examiner finds (<u>id.</u>),

> [t]he specification fails to disclose or describe any progeny resulting from such crosses, wherein said progeny could contain only a small portion of the LH321 genome, if any at all, and wherein said progeny would contain a majority of undisclosed and uncharacterized genetic material from a multitude of undisclosed and uncharacterized parents. Furthermore, no description has been provided for the progeny of such crosses with regard to even one morphological trait of said progeny containing a majority of non-LH321 genetic material.

Thus, the examiner concludes (Answer, page 13), "given the lack of an adequate written description of the claimed progeny plants, any method of using said

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[6] We note that while the examiner includes (Answer, page 13) claims 17 and 18 with claims 19, 21, 24, 30 and 31 in concluding that the claims are inadequately described, the examiner has explained (Answer, pages 12-13) the basis of this rejection as it applies to claims 19, 21, 24, 30 and 31.

progeny plants in further crosses, as claimed in claims 17-19, 21, 24, 30 and 31, would also be inadequately described.

As we understand the examiner's argument, not only does appellant have to provide a written description of the starting corn plant (LH321), but appellant also must look into the future to determine every other potential corn plant that someone may wish to cross with the LH321 inbred line, and provide written descriptive support for not only every other corn plant that could be crossed with this line, but also the resulting progeny of each cross.

As set forth in Reiffin, the purpose of the written description requirement is to "ensure that the scope of the right to exclude, as set forth in the claims does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification." Here the method of producing an inbred corn plant requires a plant of the inbred corn line LH321 be used as the starting material. To that end, to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath. The examiner has indicated that a claim to the plant of the inbred corn line LH321 is allowable, see e.g., appellant's claim 2. Therefore, in our opinion, there can be no doubt that appellant was in possession of this plant, in addition to a method of using that plant to cross with any other corn plant to produce an inbred corn plant as set forth in claims 17-19, 21, 24, 30 and 31.

In our opinion, it matters not what the other corn plants are, or what the progeny of a cross between the LH321 inbred line and some other corn plant

represents. The inventions of claims 17-19, 21, 24, 30 and 31 are drawn to the use of the LH321 inbred line as the starting material[7] to produce an inbred corn plant. In this regard, we emphasize, the claims are not drawn to a seed or plant that is the result of such a cross. Therefore, we are not persuaded by the examiner's assertion (Answer, page 11),

> [t]he product of the method of claim 31, ... would contain substantial amounts of non-LH321 genetic material [that] has not been characterized or described, because the collection of traits that it possesses has not been disclosed, and because it contains substantial amounts of non-LH321 genetic material which itself has not been described.

Accordingly, for the foregoing reasons, it is our opinion that appellant has "convey[ed] with reasonable clarity to those skilled in the art that, as of the filing date sought, [they were] in possession of the invention," Vas-Cath (emphasis omitted). Therefore, we reverse the rejection of claims 17-19, 21, 24, 30 and 31 under the written description provision of 35 U.S.C. [§] 112, first paragraph.

Claims 6 and 26-28

According to the examiner (Answer, page 13), "[c]laims 26-28 are drawn towards L321 plants further comprising a foreign gene ('transgene') which was previously isolated as a piece of DNA, and then stably inserted into the corn genome by transformation." The examiner finds, however, that "the specification does not describe identified or isolated single loci for all corn plant traits." Answer, page 14. More specifically, the examiner finds (id.), claims 26-28

[7] See Answer, page 12, wherein the examiner also recognizes that "LH321 may be used only in the initial cross...."

"broadly encompass single loci that have not been discovered or isolated." To the extent that the examiner is asserting that appellant has not provided an enabling disclosure of single loci that have not been identified, we note that to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath.

Nevertheless, it may be that the examiner's concern (Answer, page 22), is that "isolated single genes or loci that confer yield enhancement or yield stability ... have not been discovered or isolated...." In this regard, we note the examiner's assertion (id.), "[a]ppellant cannot be in possession of LH321 plants transformed with gene(s) conferring these traits." The examiner, however, provides no evidence to support the assertion that a person of ordinary skill in the art would not recognize that single loci for yield enhancement or yield stability are known in the art. In this regard, we note that appellant discloses (specification, paragraph 133), "[m]any single locus traits have been identified ... examples of these traits include, but are not limited to, ... enhanced nutritional quality, industrial usage, yield stability, and yield enhancement." It appears that the examiner has overlooked appellant's assertion that single locus traits for yield stability and yield enhancement are well known in the art. To this end, we direct the examiner's attention to, for example, United States Patent No.

5,936,145 ('145)[8], issued August 10, 1999, which is prior to the filing date of the instant application. For clarity, we reproduce claims 8, 29 and 39 of the '145 patent below:

> 8. A corn plant having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.
>
> 29. The corn plant of claim 8, further comprising a single gene conversion.
>
> 39. The single gene conversion of the corn plant of claim 29, where the gene confers enhanced yield stability.

As we understand it, claim 39 of the '145 patent, is drawn to a corn plant which comprises a single gene conversion, wherein the gene confers enhanced yield stability. Thus, contrary to the examiner's assertion it appears, for example, that a single gene that confers enhanced yield stability was known in the art prior to the filing date of the instant application. We remind the examiner "a patent need not teach, and preferably omits, what is well known in the art." Hybritech Incorporated v. Monoclonal Antibodies, Inc. 802 F.2d 1367, 1385, 231 USPQ 81, 94 (Fed. Cir. 1986).

We remind the examiner that the inquiry into whether the description requirement is met must be determined on a case-by-case basis and is a question of fact. In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). A description as filed is presumed to be adequate; unless or until sufficient evidence or reasoning to the contrary has been presented by the

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[8] We note that the assignee of the '145 patent is DeKalb Genetics Corporation. The assignee of the present application is Monsanto Company, the parent of wholly-owned subsidiary DeKalb Genetics Corporation.

examiner to rebut the presumption. See e.g., In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. Accordingly, it is the examiner who has the initial burden of establishing by a preponderance of evidence that a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. Wertheim, 541 F.2d at 263, 191 USPQ at 97. On this record, the examiner provides no evidence to support the assertion that single loci that govern, for example, yield enhancement or enhanced yield stability are not described.

Furthermore, we recognize the examiner's assertion (Answer, page 13) that "one skilled in the art would not have recognized [a]ppellant to have been in possession of the claimed hybrids or progeny plants as recited in claim[ ] 6...." As we understand it, claim 6 is drawn to a corn plant produced by growing the seed of corn inbred line LH321 further defined as comprising a gene conferring male sterility. The examiner has provided no evidence on this record as to why such a corn plant is not adequately described in appellant's specification. In this regard, we note that in the rejection under 35 U.S.C. § 112, second paragraph, the examiner suggested adding two new claims directed at (1) a method of producing a male sterile corn plant comprising transforming the plant of claim 2 with a nucleic acid molecule that confers male sterility, and (2) a male sterile corn plant produced by the method set forth above. Accordingly, we are not persuaded by the examiner's assertion that the specification does not provide an adequate written description of claim 6.

Further, we direct the examiner's attention to claim 16 of related Appeal Nos. 2005-1506 and 2004-2317, which differ from claim 6 on this record only in the variety of corn. In addition, we note that the disclosure of Appeal Nos. 2005-1506 and 2004-2317 and the instant application are substantially similar. However, in both Appeal Nos. 2005-1506 and 2004-2317 the examiner apparently found that appellant's specification provided an adequate written description of claim 16 as no rejection of this claim was made under the written description provision of 35 U.S.C. § 112, first paragraph. Accordingly, we find that the examiner has treated claim 6 in a manner that is inconsistent with the prosecution of similar claims in related applications 09/788,334 and 09/771938, which is the subject matter of Appeal Nos. 2004-1506 and 2004-2317 respectively.

For the foregoing reasons, we are not persuaded by the examiner's arguments. Accordingly, we reverse the rejection of claims 6 and 26-28 under the written description provision of 35 U.S.C. [§] 112, first paragraph.

<u>Summary</u>

For the foregoing reasons, we reverse the rejection of claims 6, 12-19, 21, 24, 26-28, 30 and 31 under the written description provision of 35 U.S.C. § 112, first paragraph.

<u>Enablement</u>:

Claims 6, 12-19, 21, 24, 26-28, 30 and 31 stand rejected under the enablement provision of 35 U.S.C. § 112, first paragraph. The examiner finds

(Answer, page 39), claims 27-30 "are broadly drawn towards inbred corn plant I015011 further defined as having a genome comprising any single locus conversion, encoding any trait; or wherein the single locus was stably inserted into a corn genome by transformation." The examiner presents several lines of argument under this heading. We take each in turn.

I. <u>Retaining the morphological fidelity of the original inbred line</u>:

According to the examiner (Answer, page 30, emphasis added), "[I]t is not clear that single loci may be introduced into the genetic background of a plant through traditional breeding, while otherwise <u>maintaining the genetic and morphological fidelity</u> of the original inbred variety...." With reference to Hunsperger, Kraft, and Eshed the examiner asserts (Answer, page 38), "[t]he rejection raises the issue of how linkage drag hampers the insertion of single genes alone into a plant by backcrossing, while recovering <u>all</u> of the original plant's genome."

We note, however, that claims 26-28 (those which are drawn to a plant transformed with one or more transgenes) do not require that the plant <u>maintain the genetic and morphological fidelity</u> of the original inbred variety. Nor do claims 26-28 require that the resultant plant retain all of the "original plant's <u>genome</u>" as a result of a backcrossing technique. As appellant explains (specification, paragraph 41, emphasis added),

> [s]ingle locus converted or conversion plant refers to plants which are developed by a plant breeding technique called backcrossing wherein <u>essentially all</u> of the desired morphological and physiological characteristics of an inbred are recovered in addition

to the single locus transferred into the inbred via the backcrossing technique or via genetic engineering.

We find nothing in the appellant's specification to indicate that the single locus converted plant retains all of the morphological and physiological traits, or all of the genome, of the parent plant in addition to the single locus transferred via the backcrossing technique. Accordingly, we disagree with the examiner's assertions to the contrary.

Further, while the examiner does not explain the basis for the rejection of claim 6 under this heading, we note as discussed supra, claim 6 is drawn to a corn plant produced by growing the seed of corn inbred line LH321 further defined as comprising a gene conferring male sterility. In this regard, we note that appellant's specification discloses (paragraph 19), "several methods of conferring genetic male sterility [that are] available [in the art]." We find no evidence in the Answer to suggest this disclosure in appellant's specification is incorrect, or insufficient. In addition, we note that the examiner's rejection of claim 6 is inconsistent with the manner in which a similar claim was treated in related applications 09/788,334 and 09/771,938, the subject matter of Appeal Nos. 2004-1506 and 2004-2317 respectively. Claim 16 of related applications 09/788,334 and 09/771,938, differs from claim 6 of the instant application only with regard to the corn variety. Nevertheless, while the disclosure in these related applications is substantially similar to the disclosure of the instant application, claim 16 was not rejected under the enablement provision of 35

U.S.C. § 112, first paragraph, in either of related applications 09/788,334 or 09/771,938.

Further, we recognize appellant's argument (Brief, page 16) that the examiner failed to establish a nexus between Hunsperger's discussion of petunias; Kraft's discussion of sugar beets; and Eshed's discussion of tomatoes, and the subject matter of the instant application - corn. Absent evidence to the contrary, we agree with appellant (id.), "the [examiner's] indication[9] that the references concerning petunias, sugar beets and tomatoes apply to corn is made without any support." That the examiner has failed to identify (Answer, page Answer, page 38) an example "in the prior art of plants in which linkage drag does not occur," does not mean that linkage drag is expected to occur in corn breeding, which according to appellant (Brief, page 16) "is extremely advanced and well known in the art." In this regard, we agree with appellant (Brief, pages 16-17), the examiner has improperly placed the burden on appellant to demonstrate that the examiner's unsupported assertion is not true. We remind the examiner, as set forth in In re Wright, 999 F.2d 1557, 1561-62, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993):

> When rejecting a claim under the enablement requirement of section 112, the PTO bears an initial burden of setting forth a reasonable explanation as to why it believes that the scope of protection provided by that claim is not adequately enabled by the description of the invention provided in the specification of the application; this includes, of course, providing sufficient reasons for doubting any assertions in the specification as to the scope of enablement.

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[9] See Answer page 38, wherein the examiner asserts "[l]inkage drag appears to be a phenomenon that occurs in all plant types."

II. Corn molecular genetic markers:

According to the examiner (Answer, page 27),

> [n]o guidance has been provided for the identification of any molecular genetic markers such as restriction fragment length polymorphisms [RFLPs] as claimed in claim 31, wherein said genetic molecular markers have been demonstrated to be inked to corn genes conferring agronomically desirable traits, or their use to breed and obtain improved corn genotypes using LH321 as the starting material.

Admittedly, we find the examiner's statement less than clear. However, as we understand it the examiner finds that the specification fails to enable claim 31 because a link between genes conferring agronomically desirable traits and RFLPs has not been established. However, as we understand claim it, claim 31 is drawn to a method of using a plant from the LH321 inbred corn line as the source of plant breeding material in the development of a corn plant in a corn plant breeding program using plant breeding techniques which are selected from the group consisting of: recurrent selection, backcrossing, pedigree breeding, RFLP enhanced selection, genetic marker enhanced selection, and transformation. As appellant discloses (specification, paragraph 3), "[t]he complexity of inheritance influences choice of the breeding method." Appellant then provides a description of various breeding methods. See e.g., specification, paragraphs 3-13. In addition, appellant discloses (specification, ape 14), several reference books wherein "[d]escriptions of other breeding methods that are commonly used for different traits and crops can be found ...." In addition, appellant provides a description of various marker genes. See e.g., specification, paragraphs 69-75. Further, appellant discloses (specification,

paragraph 91), "[f]or the relatively small number of transgenic plants that show higher levels of expression, a genetic map can be generated, primarily via conventional FRLP, PCR and SSR analysis, which identifies the approximate chromosomal location of the integrated DNA molecule." In addition, appellant provides a reference "for exemplary methodologies in this regard..." Id. Faced with this disclosure, the examiner provides no evidence to support his assertion that appellant's specification does not provide an enabling disclosure of the invention set forth in claim 31.

As set forth in In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971), the burden is on

> the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure.

On this record, we find only the examiner's unsupported conclusions as to why the specification does not enable the claimed invention. We remind the examiner that nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples. Marzocchi, 439 F.2d at 223, 169 USPQ at 369. In the absence of an evidentiary basis to support the rejection, the examiner has not sustained his initial burden of establishing a prima facie case of non-enablement. In this regard, we note that the burden of proof does not shift to appellant until

the examiner first meets his burden. Marzocchi, 439 F.2d at 223-224, 169 USPQ at 369-370.

Accordingly, we are not persuaded by the examiner's comments.

III. Non-exemplified breeding partners:

The examiner finds (Answer, page 27), "[n]o guidance has been provided regarding the morphological or genetic compositions of a multitude of non-exemplified breeding partners for crossing with LH321...." According to the examiner this is true whether a single cross is preformed to produce a hybrid corn plant as claimed in claims 12-16, or multiple crosses with non-LH321 parents over multiple generations as claimed in claims 19, 21, 24, 30 and 31, with or without multiple non-disclosed parents.[10]

Claims 12-16:

As discussed supra, the examiner has interpreted these claims as directed to the product of a single cross of a LH321 plant and a non-LH321 plant. See Answer, page 5, and 27. Accordingly, as we understand this record, claims 12-16 are drawn to F_1 hybrid seed, plant, or plant parts. The claims do not require the hybrid to express any particular morphological or physiological

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[10] We note that the examiner includes claim 14 in a discussion of "multiple crosses with non-LH321 parents over multiple generations." However, as we understand the claim, claim 14 is drawn to the seed produced by growing the corn plant of claim 13 and harvesting the resultant corn seed. Accordingly, it appears that the examiner has inadvertently included claim 14 together with claims 19, 21, 24, 30 and 31. As we understand claim 14, it should have been included with the rejection of claims 12, 13, 15 and 16. See e.g., Answer, page 5, wherein the examiner's treatment of claims 12-16 together as "drawn towards any hybrid corn seed produced by the process of crossing the inbred corn plant LH321 with any second, distinct, inbred corn plant; and any hybrid corn plant produced by growing said hybrid corn seed (claims 12-16). Accordingly, we have considered the examiner's argument regarding claim 14 together with claims 12, 13, 15 and 16.

characteristic. Nor do the claims require that a particular non-LH321 corn variety be used. All that is required by the claims is that the F_1 hybrid has one parent that is a plant of corn variety LH321.

Since the examiner has indicated that the seed and the plant of the inbred line LH321 are allowable (see claims 1 and 2), there can be no doubt that the specification provides an adequate written description of this inbred corn line. In addition, the examiner recognizes (Answer, page 7) that appellant's specification describes four exemplary hybrids wherein one parent was a plant of the inbred corn line LH321, see e.g., specification, pages 31-33. Accordingly, it is unclear to this merits panel what additional enabling description is necessary. In our opinion, appellant's specification provides an enabling description of F_1 hybrids wherein one parent is a corn plant of the LH321 inbred line.

Claims 17-19, 21, 24, 30 and 31:

We understand these claims to be drawn to methods of producing plants derived from LH321. Stated differently, the claims are drawn to methods of using LH321 inbred corn plants as the starting material to produce other inbred lines. In our opinion, it matters not what the other corn plants are, or what the progeny of a cross between the LH321 inbred line and some other corn plant represents. The inventions of claims 17-19, 21, 24, 30 and 31 are drawn to the use of the LH321 inbred line as the starting material[11] to produce an inbred corn plant. In this regard, we emphasize, these claims are not drawn to a seed or

[11] See Answer, page 12, wherein the examiner also recognizes that "LH321 may be used only in the initial cross...."

plant that is the result of such a cross. The examiner has provided no evidence on this record that person of ordinary skill in the art could not produce another inbred line, which uses a corn plant of the LH321 inbred line as the starting material. Therefore, we are not persuaded by the examiner's unsupported assertions to the contrary.

Accordingly, for the foregoing reasons, we reverse the rejection of claims 6, 12-19, 21, 24, 26-28, 30 and 31 under the enablement provision of 35 U.S.C. § 112, first paragraph.

SUMMARY

We reverse the rejection of claims 6 and 26-28 under 35 U.S.C. § 112, second paragraph.

We reverse the rejection of claims 6, 12-19, 21, 24, 26-28, 30 and 31 under the written description provision of 35 U.S.C. § 112, first paragraph.

We reverse the rejection of claims 6, 12-19, 21, 24, 26-28, 30 and 31 under the enablement provision of 35 U.S.C. § 112, first paragraph.

We do not reach the merits of the rejection of claim 8 under 35 U.S.C. § 112, second paragraph, which was not presented for our review in this appeal.

REVERSED

Toni R. Scheiner Administrative Patent Judge	)
	) BOARD OF PATENT
Donald E. Adams Administrative Patent Judge	) APPEALS AND
	) INTERFERENCES
Lora M. Green Administrative Patent Judge	)

ROBERT E. HANSON
FULBRIGHT & JAWORSKI L.L.P.
600 CONGRESS AVENUE
SUITE 2400
AUSTIN TX 78701

Notice of References Cited	Application/Control No. 10/000,311	Applicant(s)/Patent Under Reexamination Appeal No. 2004-1968	
	Examiner BPAI	Art Unit	Page of

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-5,936,145	08-1999	Bradbury	
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

US005936145A

United States Patent [19]

Bradbury

[11] **Patent Number:** 5,936,145

[45] **Date of Patent:** Aug. 10, 1999

[54] INBRED CORN PLANT 87DIA4 AND SEEDS THEREOF

[75] Inventor: Peter J. Bradbury, Madison, Wis.

[73] Assignee: DeKalb Genetics Corporation, DeKalb, Ill.

[21] Appl. No.: 09/017,996

[22] Filed: Feb. 3, 1998

Related U.S. Application Data

[60] Provisional application No. 60/037,305, Feb. 5, 1997.

[51] Int. Cl.^{6} A01H 5/00; A01H 4/00; A01H 1/00; C12N 5/04

[52] U.S. Cl. 800/320.1; 800/298; 800/275; 800/271; 800/301; 800/302; 800/303; 435/412; 435/424; 435/430; 435/430.1

[58] Field of Search 800/320.1, 298, 800/275, 271, 303, 274, 302; 435/172.3, 172.1, 412, 424, 430, 430.1

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Primary Examiner—Gary Benzion
Attorney, Agent, or Firm—Arnold, White & Durkee

[57] ABSTRACT

According to the invention, there is provided an inbred corn plant designated 87DIA4. This invention thus relates to the plants, seeds and tissue cultures of the inbred corn plant 87DIA4, and to methods for producing a corn plant produced by crossing the inbred plant 87DIA4 with itself or with another corn plant, such as another inbred. This invention further relates to corn seeds and plants produced by crossing the inbred plant 87DIA4 with another corn plant, such as another inbred, and to crosses with related species. This invention further relates to the inbred and hybrid genetic complements of the inbred corn plant 87DIA4, and also to the RFLP and genetic isozyme typing profiles of inbred corn plant 87DIA4.

39 Claims, No Drawings

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INBRED CORN PLANT 87DIA4 AND SEEDS THEREOF

The present application claims the priority of co-pending U.S. Provisional Patent Application Serial No. 60/037,305, filed Feb. 5, 1997, the entire disclosure of which is incorporated herein by reference without disclaimer.

BACKGROUND OF THE INVENTION

I. Technical Field of the Invention

The present invention relates to the field of corn breeding. In particular, the invention relates to the inbred corn seed and plant designated 87DIA4, and derivatives and tissue cultures of such inbred plant.

II. Description of the Background Art

The goal of field crop breeding is to combine various desirable traits in a single variety/hybrid. Such desirable traits include greater yield, better stalks, better roots, resistance to insecticides, herbicides, pests, and disease, tolerance to heat and drought, reduced time to crop maturity, better agronomic quality, and uniformity in germination times, stand establishment, growth rate, maturity, and fruit size.

Breeding techniques take advantage of a plant's method of pollination. There are two general methods of pollination: a plant self-pollinates if pollen from one flower is transferred to the same or another flower of the same plant. A plant cross-pollinates if pollen comes to it from a flower on a different plant.

Corn plants (*Zea mays L.*) can be bred by both self-pollination and cross-pollination. Both types of pollination involve the corn plant's flowers. Corn has separate male and female flowers on the same plant, located on the tassel and the ear, respectively. Natural pollination occurs in corn when wind blows pollen from the tassels to the silks that protrude from the tops of the ear shoot.

Plants that have been self-pollinated and selected for type over many generations become homozygous at almost all gene loci and produce a uniform population of true breeding progeny, a homozygous plant. A cross between two such homozygous plants produce a uniform population of hybrid plants that are heterozygous for many gene loci. Conversely, a cross of two plants each heterozygous at a number of gene loci produces a population of hybrid plants that differ genetically and are not uniform. The resulting non-uniformity makes performance unpredictable.

The development of uniform corn plant hybrids requires the development of homozygous inbred plants, the crossing of these inbred plants, and the evaluation of the crosses. Pedigree breeding and recurrent selection are examples of breeding methods used to develop inbred plants from breeding populations. Those breeding methods combine the genetic backgrounds from two or more inbred plants or various other broad-based sources into breeding pools from which new inbred plants are developed by selfing and selection of desired phenotypes. The new inbreds are crossed with other inbred plants and the hybrids from these crosses are evaluated to determine which of those have commercial potential.

The pedigree breeding method for single-gene traits involves crossing two genotypes. Each genotype can have one or more desirable characteristics lacking in the other; or, each genotype can complement the other. If the two original parental genotypes do not provide all of the desired characteristics, other genotypes can be included in the breeding population. Superior plants that are the products of these crosses are selfed and selected in successive generations. Each succeeding generation becomes more homogeneous as a result of self-pollination and selection. Typically, this method of breeding involves five or more generations of selfing and selection: $S_1 \rightarrow S_2$; $S_2 \rightarrow S_3$; $S_3 \rightarrow S_4$; $S_4 \rightarrow S_5$, etc. After at least five generations, the inbred plant is considered genetically pure.

Backcrossing can also be used to improve an inbred plant. Backcrossing transfers a specific desirable trait from one inbred or other source to an inbred that lacks that trait. This can be accomplished for example by first crossing a superior inbred (A) (recurrent parent) to a donor inbred (non-recurrent parent), which carries the appropriate gene(s) for the trait in question. The progeny of this cross are then mated back to the superior recurrent parent (A) followed by selection in the resultant progeny for the desired trait to be transferred from the non-recurrent parent. After five or more backcross generations with selection for the desired trait, the progeny are heterozygous for loci controlling the characteristic being transferred, but are like the superior parent for most or almost all other genes. The last backcross generation would be selfed to give pure breeding progeny for the gene(s) being transferred.

A single cross hybrid corn variety is the cross of two inbred plants, each of which has a genotype which complements the genotype of the other. The hybrid progeny of the first generation is designated F_1. Preferred F_1 hybrids are more vigorous than their inbred parents. This hybrid vigor, or heterosis, is manifested in many polygenic traits, including markedly improved higher yields, better stalks, better roots, better uniformity and better insect and disease resistance. In the development of hybrids only the F_1 hybrid plants are sought. An F_1 single cross hybrid is produced when two inbred plants are crossed. A double cross hybrid is produced from four inbred plants crossed in pairs (A×B and C×D) and then the two F_1 hybrids are crossed again (A×B)×(C×D).

The development of a hybrid corn variety involves three steps: (1) the selection of plants from various germplasm pools; (2) the selfing of the selected plants for several generations to produce a series of inbred plants, which, although different from each other, each breed true and are highly uniform; and (3) crossing the selected inbred plants with unrelated inbred plants to produce the hybrid progeny (F_1). During the inbreeding process in corn, the vigor of the plants decreases. Vigor is restored when two unrelated inbred plants are crossed to produce the hybrid progeny (F_1). An important consequence of the homozygosity and homogeneity of the inbred plants is that the hybrid between any two inbreds is always the same. Once the inbreds that give a superior hybrid have been identified, hybrid seed can be reproduced indefinitely as long as the homogeneity of the inbred parents is maintained. Conversely, much of the hybrid vigor exhibited by F_1 hybrids is lost in the next generation (F_2). Consequently, seed from hybrid varieties is not used for planting stock. It is not generally beneficial for farmers to save seed of F_1 hybrids. Rather, farmers purchase F_1 hybrid seed for planting every year.

North American farmers plant over 70 million acres of corn at the present time and there are extensive national and international commercial corn breeding programs. A continuing goal of these corn breeding programs is to develop high-yielding corn hybrids that are based on stable inbred plants that maximize the amount of grain produced and minimize susceptibility to environmental stresses. To accomplish this goal, the corn breeder must select and develop superior inbred parental plants for producing hybrids.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a corn plant designated 87DIA4. Also provided are corn plants having all the physiological and morphological characteristics of corn plant 87DIA4.

The inbred corn plant of the invention may further comprise, or have, a cytoplasmic factor that is capable of conferring male sterility. Parts of the corn plant of the present invention are also provided, such as, e.g., pollen obtained from an inbred plant and an ovule of the inbred plant.

The invention also concerns seed of the corn plant 87DIA4, which has been deposited with the ATCC. The invention thus provides inbred corn seed designated 87DIA4, and having ATCC Accession No. 203192.

The inbred corn seed of the invention may be provided as an essentially homogeneous population of inbred corn seed designated 87DIA4.

Essentially homogeneous populations of inbred seed are those that consist essentially of the particular inbred seed, and are generally purified free from substantial numbers of other seed, so that the inbred seed forms between about 90% and about 100% of the total seed, and preferably, between about 95% and about 100% of the total seed. Most preferably, an essentially homogeneous population of inbred corn seed will contain between about 98.5%, 99%, 99.5% and about 100% of inbred seed, as measured by seed grow outs.

In any event, even if a population of inbred corn seed was found, for some reason, to contain about 50%, or even about 20% or 15% of inbred seed, this would still be distinguished from the small fraction of inbred seed that may be found within a population of hybrid seed, e.g., within a bag of hybrid seed. In such a bag of hybrid seed offered for sale, the Governmental regulations require that the hybrid seed be at least about 95% of the total seed. In the practice of the present invention, the hybrid seed generally forms at least about 97% of the total seed. In the most preferred practice of the invention, the female inbred seed that may be found within a bag of hybrid seed will be about 1% of the total seed, or less, and the male inbred seed that may be found within a bag of hybrid seed will be negligible, i.e., will be on the order of about a maximum of 1 per 100,000, and usually less than this value.

The population of inbred corn seed of the invention is further particularly defined as being essentially free from hybrid seed. The inbred seed population may be separately grown to provide an essentially homogeneous population of inbred corn plant designated 87DIA4.

In another aspect, the present invention provides for single gene converted plants of 87DIA4. The single transferred gene may preferably be a dominant or recessive allele. Preferably, the single transferred gene will confer such traits as male sterility, herbicide resistance, insect resistance, resistance for bacterial, fungal, or viral disease, male fertility, enhanced nutritional quality, and industrial usage. The single gene may be a naturally occurring maize gene or a transgene introduced through genetic engineering techniques.

In another aspect, the present invention provides a tissue culture of regenerable cells of inbred corn plant 87DIA4. The tissue culture will preferably be capable of regenerating plants having the physiological and morphological characteristics of the foregoing inbred corn plant, and of regenerating plants having substantially the same genotype as the foregoing inbred corn plant. Preferably, the regenerable cells in such tissue cultures will be embryos, protoplasts, meristematic cells, callus, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks or stalks. Still further, the present invention provides corn plants regenerated from the tissue cultures of the invention.

In yet another aspect, the present invention provides processes for preparing corn seed or plants, which processes generally comprise crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is the inbred corn plant designated 87DIA4. These processes may be further exemplified as processes for preparing hybrid corn seed or plants, wherein a first inbred corn plant is crossed with a second, distinct inbred corn plant to provide a hybrid that has, as one of its parents, the inbred corn plant 87DIA4.

In a preferred embodiment, crossing comprises planting, in pollinating proximity, seeds of the first and second parent corn plant, and preferably, seeds of a first inbred corn plant and a second, distinct inbred corn plant; cultivating or growing the seeds of said first and second parent corn plants into plants that bear flowers; emasculating the male flowers of the first or second parent corn plant, i.e., treating the flowers so as to prevent pollen production, in order to produce an emasculated parent corn plant; allowing natural cross-pollination to occur between the first and second parent corn plants; and harvesting the seeds from the emasculated parent corn plant. Where desired, the harvested seed is grown to produce a corn plant or hybrid corn plant.

The present invention also provides corn seed and plants produced by a process that comprises crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is the inbred corn plant designated 87DIA4. In one embodiment, corn plants produced by the process are first generation (F_1) hybrid corn plants produced by crossing an inbred in accordance with the invention with another, distinct inbred. The present invention further contemplates seed of an F_1 hybrid corn plant.

In certain exemplary embodiments, the invention provides an F_1 hybrid corn plant and seed thereof, which hybrid corn plant is designated 4033843, having 871DIA4 as one inbred parent.

In yet a further aspect, the invention provides an inbred genetic complement of the corn plant designated 87DIA4. The phrase "genetic complement" is used to refer to the aggregate of nucleotide sequences, the expression of which sequences defines the phenotype of, in the present case, a corn plant, or a cell or tissue of that plant. An inbred genetic complement thus represents the genetic make up of an inbred cell, tissue or plant, and a hybrid genetic complement represents the genetic make up of a hybrid cell, tissue or plant. The invention thus provides corn plant cells that have a genetic complement in accordance with the inbred corn plant cells disclosed herein, and plants, seeds and diploid plants containing such cells.

Plant genetic complements may be assessed by genetic marker profiles, and by the expression of phenotypic traits that are characteristic of the expression of the genetic complement, e.g., isozyme typing profiles. Thus, such corn plant cells may be defined as having an RFLP genetic marker profile in accordance with the profile shown in Table 8, or a genetic isozyme typing profile in accordance with the profile shown in Table 9, or having both an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 8 and 9.

In another aspect, the present invention provides hybrid genetic complements, as represented by corn plant cells, tissues, plants and seeds, formed by the combination of a haploid genetic complement of an inbred corn plant of the invention with a haploid genetic complement of a second corn plant, preferably, another, distinct inbred corn plant. In another aspect, the present invention provides a corn plant regenerated from a tissue culture that comprises a hybrid genetic complement of this invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. DEFINITIONS

Barren Plants: Plants that are barren, i.e., lack an ear with grain, or have an ear with only a few scattered kernels.

Cg: *Colletotrichum graminicola* rating. Rating times 10 is approximately equal to percent total plant infection.

CLN: Corn Lethal Necrosis (combination of Maize (Chlorotic Mottle Virus and Maize Dwarf Mosaic virus) rating: numerical ratings are based on a severity scale where 1=most resistant to 9=susceptible.

Cn: *Corynebacterium nebraskense* rating. Rating times 10 is approximately equal to percent total plant infection.

Cz: *Cercospora zeae-maydis* rating. Rating times 10 is approximately equal to percent total plant infection.

Dgg: *Diatraea grandiosella* girdling rating (values are percent plants girdled and stalk lodged).

Dropped Ears: Ears that have fallen from the plant to the ground.

Dsp: Diabrotica species root ratings (1=least affected to 9=severe pruning).

Ear-Attitude: The attitude or position of the ear at harvest scored as upright, horizontal, or pendant.

Ear-Cob Color: The color of the cob, scored as white, pink, red, or brown.

Ear-Cob Diameter: The average diameter of the cob measured at the midpoint.

Ear-Cob Strength: A measure of mechanical strength of the cobs to breakage, scored as strong or weak.

Ear-Diameter: The average diameter of the ear at its midpoint.

Ear-Dry Husk Color: The color of the husks at harvest scored as buff, red, or purple.

Ear-Fresh Husk The color of the husks 1 to 2 weeks after pollination scored as Color: green, red, or purple.

Ear-Husk Bract: The length of an average husk leaf scored as short, medium, or long.

Ear-Husk Cover: The average distance from the tip of the ear to the tip of the husks. Minimum value no less than zero.

Ear-Husk Opening: An evaluation of husk tightness at harvest scored as tight, intermediate, or open.

Ear-Length: The average length of the ear.

Ear-Number Per The average number of ears per plant. Stalk:

Ear-Shank The average number of internodes on the ear shank. Internodes:

Ear-Shank Length: The average length of the ear shank.

Ear-Shelling Percent: The average of the shelled grain weight divided by the sum of the shelled grain weight and cob weight for a single ear.

Ear-Silk Color: The color of the silk observed 2 to 3 days after silk emergence scored as green-yellow, yellow, pink, red, or purple.

Ear-Taper (Shape): The taper or shape of the ear scored as conical, semi-conical, or cylindrical.

Ear-Weight: The average weight of an ear.

Early Stand: The percent of plants that emerge from the ground as determined in the early spring.

ER: Ear rot rating (values approximate percent ear rotted).

Final Stand Count: The number of plants just prior to harvest.

GDUs to Shed: The number of growing degree units (GDUs) or heat units required for an inbred line or hybrid to have approximately 50 percent of the plants shedding pollen as measured from time of planting. Growing degree units are calculated by the Barger Method, where the heat units for a 24-hour period are calculated as GDUs=[Maximum daily temperature+Minimum daily temperature)/2]−50. The highest maximum daily temperature used is 86 degrees Fahrenheit and the lowest minimum temperature used is 50 degrees Fahrenheit. GDUs to shed is then determined by summing the individual daily values from planting date to the date of 50 percent pollen shed.

GDUs to Silk: The number of growing degree units for an inbred line or hybrid to have approximately 50 percent of the plants with silk emergence as measured from time of planting. Growing degree units are calculated by the same methodology as indicated in the GDUs to shed definition.

Hc2: *Helminthosporium carbonum* race 2 rating. Rating times 10 is approximately equal to percent total plant infection.

Hc3: *Helminthosporium carbonum* race 3 rating. Rating times 10 is approximately equal to percent total plant infection.

Hm: *Helminthosporium maydis* race 0 rating. Rating times 10 is approximately equal to percent total plant infection.

Ht1: *Helminthosporium turcicum* race 1 rating. Rating times 10 is approximately equal to percent total plant infection.

Ht2: *Helminthosporium turcicum* race 2 rating. Rating times 10 is approximately equal to percent total plant infection.

HtG: +=Presence of Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection. −=Absence of a Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection. +/−=Segregation of a Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection.

Kernel-Aleurone The color of the aleurone scored as white, pink, tan, brown, Color: bronze, red, purple, pale purple, colorless, or variegated.

Kernel-Cap Color: The color of the kernel cap observed at dry stage, scored as white, lemon-yellow, yellow or orange.

Kernel-Endosperm The color of the endosperm scored as white, pale yellow, or Color: yellow.

Kernel-Endosperm The type of endosperm scored as normal, waxy, or opaque. Type:

Kernel-Grade: The percent of kernels that are classified as rounds.

Kernel-Length: The average distance from the cap of the kernel to the pedicel.

Kernel-Number Per The average number of kernels in a single row. Row:

Kernel-Pericarp The color of the pericarp scored as colorless, red-white crown, tan, Color: bronze, brown, light red, cherry red, or variegated.

Kernel-Row The direction of the kernel rows on the ear scored as straight, Direction: slightly curved, spiral, or indistinct (scattered).

Kernel-Row Number: The average number of rows of kernels on a single ear.

Kernel-Side Color: The color of the kernel side observed at the dry stage, scored as white, pale yellow, yellow, orange, red, or brown.

Kernel-Thickness: The distance across the narrow side of the kernel.

Kernel-Type: The type of kernel scored as dent, flint, or intermediate.

Kernel-Weight: The average weight of a predetermined number of kernels.

Kernel-Width: The distance across the flat side of the kernel.

Kz: *Kabatiella zeae* rating. Rating times 10 is approximately equal to percent total plant infection.

Leaf-Angle: Angle of the upper leaves to the stalk scored as upright (0 to 30 degrees), intermediate (30 to 60 degrees), or lax (60 to 90 degrees).

Leaf-Color: The color of the leaves 1 to 2 weeks after pollination scored as light green, medium green, dark green, or very dark green.

Leaf-Length: The average length of the primary ear leaf.

Leaf-Longitudinal A rating of the number of longitudinal creases on the leaf surface 1 Creases: to 2 weeks after pollination. Creases are scored as absent, few, or many.

Leaf-Marginal A rating of the waviness of the leaf margin 1 to 2 weeks after Waves: pollination. Rated as none, few, or many.

Leaf-Number: The average number of leaves of a mature plant. Counting begins with the cotyledonary leaf and ends with the flag leaf.

Leaf-Sheath A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks Anthocyanin: after pollination, scored as absent, basal-weak, basal-strong, weak or strong.

Leaf-Sheath A rating of the pubescence of the leaf sheath. Ratings are taken 1 Pubescence: to 2 weeks after pollination and scored as light, medium, or heavy.

Leaf-Width: The average width of the primary ear leaf measured at its widest point.

LSS: Late season standability (values times 10 approximate percent plants lodged in disease evaluation plots).

Moisture: The moisture of the grain at harvest.

On1: *Ostrinia nubilalis* 1st brood rating (1=resistant to 9=susceptible).

On2: *Ostrinia nubilalis* 2nd brood rating (1=resistant to 9=susceptible).

Relative Maturity: A maturity rating based on regression analysis. The regression analysis is developed by utilizing check hybrids and their previously established day rating versus actual harvest moistures. Harvest moisture on the hybrid in question is determined and that moisture value is inserted into the regression equation to yield a relative maturity.

Root Lodging: Root lodging is the percentage of plants that root lodge. A plant is counted as root lodged if a portion of the plant leans from the vertical axis by approximately 30 degrees or more.

Seedling Color: Color of leaves at the 6 to 8 leaf stage.

Seedling Height: Plant height at the 6 to 8 leaf stage.

Seedling Vigor: A visual rating of the amount of vegetative growth on a 1 to 9 scale, where 1 equals best. The score is taken when the average entry in a trial is at the fifth leaf stage.

Selection Index: The selection index gives a single measure of hybrid's worth based on information from multiple traits. One of the traits that is almost always included is yield. Traits may be weighted according to the level of importance assigned to them.

Sr: *Sphacelotheca reiliana* rating is actual percent infection.

Stalk-Anthocyanin: A rating of the amount of anthocyanin pigmentation in the stalk. The stalk is rated 1 to 2 weeks after pollination as absent, basal-weak, basal-strong, weak, or strong.

Stalk-Brace Root The color of the brace roots observed 1 to 2 weeks after pollination Color: as green, red, or purple.

Stalk-Diameter: The average diameter of the lowest visible internode of the stalk.

Stalk-Ear Height: The average height of the ear measured from the ground to the point of attachment of the ear shank of the top developed ear to the stalk.

Stalk-Internode The direction of the stalk internode observed after pollination as Direction: straight or zigzag.

Stalk-Internode The average length of the internode above the primary ear. Length:

Stalk Lodging: The percentage of plants that did stalk lodge. Plants are counted as stalk lodged if the plant is broken over or off below the ear.

Stalk-Nodes With The average number of nodes having brace roots per plant. Brace Roots:

Stalk-Plant Height: The average height of the plant as measured from the soil to the tip of the tassel.

Stalk-Tillers: The percent of plants that have tillers. A tiller is defined as a secondary shoot that has developed as a tassel capable of shedding pollen.

Staygreen: Staygreen is a measure of general plant health near the time of black layer formation (physiological maturity). It is usually recorded at the time the ear husks of most entries within a trial have turned a mature color. Scoring is on a 1 to 9 basis where 1 equals best.

STR: Stalk rot rating (values represent severity rating of 1=25 percent of inoculated internode rotted to 9=entire stalk rotted and collapsed).

SVC: Southeastern Virus Complex combination of Maize Chlorotic Dwarf Virus and Maize Dwarf Mosaic Virus) rating; numerical ratings are based on a severity scale where 1=most resistant to 9=susceptible (1988 reactions are largely Maize Dwarf Mosaic Virus reactions).

Tassel-Anther Color: The color of the anthers at 50 percent pollen shed scored as green-yellow, yellow, pink, red, or purple.

Tassel-Attitude: The attitude of the tassel after pollination scored as open or compact.

Tassel-Branch Angle: The angle of an average tassel branch to the main stem of the tassel scored as upright (less than 30 degrees), intermediate (30 to 45 degrees), or lax (greater than 45 degrees).

Tassel-Branch The average number of primary tassel branches. Number:

Tassel-Glume Band: The closed anthocyanin band at the base of the glume scored as present or absent.

Tassel-Glume Color: The color of the glumes at 50 percent shed scored as green, red, or purple.

Tassel-Length: The length of the tassel measured from the base of the bottom tassel branch to the tassel tip.

Tassel-Peduncle The average length of the tassel peduncle, measured from the base Length: of the flag leaf to the base of the bottom tassel branch.

Tassel-Pollen Shed: A visual rating of pollen shed determined by tapping the tassel and observing the pollen flow of approximately five plants per entry. Rated on a 1 to 9 scale where 9=sterile, 1=most pollen.

Tassel-Spike Length: The length of the spike measured from the base of the top tassel branch to the tassel tip.

Test Weight: The measure of the weight of the grain in pounds for a given volume (bushel) adjusted to 15.5 percent moisture.

Yield: Yield of grain at harvest adjusted to 15.5 percents moisture.

II. OTHER DEFINITIONS

Allele is any of one or more alternative forms of a gene, all of which alleles relate to one trait or characteristic. In a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes.

Backcrossing is a process in which a breeder repeatedly crosses hybrid progeny back to one of the parents, for example, a first generation hybrid (F_1) with one of the parental genotypes of the F1 hybrid.

Chromatography is a technique wherein a mixture of dissolved substances are bound to a solid support followed by passing a column of fluid across the solid support and varying the composition of the fluid. The components of the mixture are separated by selective elution.

Crossing refers to the mating of two parent plants.

Cross-pollination refers to fertilization by the union of two gametes from different plants.

Diploid refers to a cell or organism having two sets of chromosomes.

Electrophoresis is a process by which particles suspended in a fluid are moved under the action of an electrical field, and thereby separated according to their charge and molecular weight. This method of separation is well known to those skilled in the art and is typically applied to separating various forms of enzymes and of DNA fragments produced by restriction endonucleases.

Emasculate refers to the removal of plant male sex organs.

Enzymes are organic catalysts that can exist in various forms called isozymes.

F_1 Hybrid refers to the first generation progeny of the cross of two plants.

Genetic Complement refers to an aggregate of nucleotide sequences, the expression of which sequences defines the phenotype in corn plants, or components of plants including cells or tissue.

Genotype refers to the genetic constitution of a cell or organism.

Haploid refers to a cell or organism having one set of the two sets of chromosomes in a diploid.

Isozymes are one of a number of enzymes which catalyze the same reaction(s) but differ from each other, e.g., in primary structure and/or electrophoretic mobility. The differences between isozymes are under single gene, codominant control. Consequently, electrophoretic separation to produce band patterns can be equated to different alleles at the DNA level. Structural differences that do not alter charge cannot be detected by this method.

Isozyme typing profile refers to a profile of band patterns of isozymes separated by electrophoresis that can be equated to different alleles at the DNA level.

Linkage refers to a phenomenon wherein alleles on the same chromosome tend to segregate together more often than expected by chance if their transmission was independent.

Marker is a readily detectable phenotype, preferably inherited in codominant fashion (both alleles at a locus in a diploid heterozygote are readily detectable), with no environmental variance component, i.e., heritability of 1.

87DIA4 refers to the corn plant from which seeds having ATCC Accession No. 203192 were obtained, as well as plants grown from those seeds.

Phenotype refers to the detectable characteristics of a cell or organism, which characteristics are the manifestation of gene expression.

Quantitative Trait Loci (QTL) refer to genetic loci that control to some degree numerically representable traits that are usually continuously distributed.

Regeneration refers to the development of a plant from tissue culture.

RFLP genetic marker profile refers to a profile of band patterns of DNA fragment lengths typically separated by agarose gel electrophoresis, after restriction endonuclease digestion of DNA.

Self-pollination refers to the transfer of pollen from the anther to the stigma of the same plant.

Single Gene Converted (Conversion) Plant refers to plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single gene transferred into the inbred via the backcrossing technique.

Tissue Culture refers to a composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

III. INBRED CORN PLANT 87DIA4

In accordance with one aspect of the present invention, there is provided a novel inbred corn plant, designated 87DIA4. Inbred corn plant 87DIA4 is a yellow, dent corn inbred that can be compared to inbred corn plants 2FACC, 3AZA1, and AQA3, all of which are proprietary inbreds of DEKALB Genetics Corporation. 87DIA4 differs significantly (at the 1%, 5%, or 10% level) from these inbred lines in several aspects (Table 1, Table 2, and Table 3).

TABLE 1

COMPARISON OF 87DIA4 WITH 2FACC

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
2FACC	0.4	0.6	29.5	62.0	23.9	67.1	1.3	1482.6	1481.5	5.8	77.4
DIFF	-0.1	-0.5	-5.2	0.4	-6.1	-10.1	-1.1	-119.5	-124.4	2.4	-12.3
# LOC	15	13	8	15	14	8	14	8	8	13	13
P VALUE	0.88	0.65	0.00**	0.84	0.00**	0.00**	0.40	0.00**	0.00**	0.36	0.01*

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

TABLE 2

COMPARISON OF 87DIA4 WITH 3AZA1

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
3AZA1	1.1	1.6	23.6	62.0	15.9	58.4	0.1	1322.6	1321.2	8.4	41.1
DIFF	-0.8	-1.5	0.7	0.4	1.9	-1.4	0.1	40.5	35.9	-0.2	24.0
# LOC	15	13	8	15	14	8	14	8	8	13	13
P VALUE	0.41	0.19	0.66	0.84	0.13	0.48	0.94	0.00**	0.03*	0.94	0.00**

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

TABLE 3

COMPARISON OF 87DIA4 WITH AQA3

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
AQA3	0.4	0.5	25.9	62.7	14.4	58.1	1.0	1356.2	1348.6	15.2	35.7
DIFF	-0.1	-0.4	-1.6	-0.3	3.3	-1.1	-0.8	6.9	8.5	-7.0	29.4

TABLE 3-continued

COMPARISON OF 87DIA4 WITH AQA3

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
# LOC	15	13	8	15	14	8	14	8	8	10	13
P VALUE	0.86	0.72	0.34	0.86	0.00**	0.58	0.56	0.64	0.63	0.01*	0.00**

Significance levels are indicated as:
+ = 10 percent.
* = 5 percent.
** = 1 percent.
Legend Abbreviations:
BARREN % - Barren Plants (percent)
DROP % - Dropped Ears (percent)
EHT INCH - Ear Height (inches)
FINAL - Final Stand
MST % - Moisture (percent)
PHT INCH - Plant Height (inches)
RTL % - Root Lodging (percent)
SHED GDU - GDUs to Shed
SILK GDU - GDUS to Silk
STL % - Stalk Lodging (percent)
YLD BU - Yield (bushels/acre)

A. ORIGIN AND BREEDING HISTORY

Inbred plant 87DIA4 was derived from the cross between a line derived from 2FACC and 3AZA1.

87DIA4 shows uniformity and stability within the limits of environmental influence for the traits described hereinafter in Table 4. 87DIA4 has been self-pollinated and ear-rowed a sufficient number of generations with careful attention paid to uniformity of plant type to ensure homozygosity and phenotypic stability. No variant traits have been observed or are expected in 87DIA4.

A deposit of 2500 seeds of plant designated 87DIA4 has been made with the American Type Culture Collection (ATCC), Rockville Pike, Bethesda, Md. on Sep. 11, 1998. Those deposited seeds have been assigned Accession No. 203192. The deposit was made in accordance with the terms and provisions of the Budapest Treaty relating to deposit of microorganisms and is made for a term of at least thirty (30) years and at least five (05) years after the most recent request for the furnishing of a sample of the deposit was received by the depository, or for the effective term of the patent, whichever is longer, and will be replaced if it becomes non-viable during that period.

Inbred corn plants can be reproduced by planting such inbred seeds, growing the resulting corn plants under self-pollinating or sib-pollinating conditions with adequate isolation using standard techniques well known to an artisan skilled in the agricultural arts. Seeds can be harvested from such a plant using standard, well known procedures.

The origin and breeding history of inbred plant 87DIA4 can be summarized as follows:

Summer 1988 The cross 2FACC and AQA3 was made. Both inbreds are proprietary to DEKALB Genetics Corporation.

Winter 1988 S0 seed was grown (nursery row 67-51).

Summer 1989 S1 seed was grown (nursery rows 4-25 to 4-38).

Winter 1989 S2 seed was grown ear-to-row (nursery row 649-62).

Summer 1990 S3 seed was grown ear-to-row (nursery row 130-15).

Winter 1990 S4 seed was grown ear-to-row (nursery row C23-23).

Summer 1991 S5 seed was grown ear-to-row (nursery row 222-67).

Summer 1992 S6 seed was grown ear-to-row (nursery row 418-56).

Summer 1993 S7 seed was grown ear-to-row (nursery rows 346-32 to 346-39). Seed from all rows was bulked to form 87DIA4.

B. PHENOTYPIC DESCRIPTION

In accordance with another aspect of the present invention, there is provided a corn plant having the physiological and morphological characteristics of corn plant 87DIA4. A description of the physiological and morphological characteristics of corn plant 87DIA114 is presented in Table 4.

TABLE 4

MORPHOLOGICAL TRAITS FOR THE 87DIA4 PHENOTYPE

CHARACTERISTIC	87DIA4	2FACC	3AZA1	AQA3
1. STALK				
Diameter (width) cm	1.9	2.2	2.0	2.2
Anthocyanin	Absent	Absent	Absent	Absent
Nodes with Brace Roots	1.4	1.9	2.0	1.5
Brace Root Color	Red	Purple	—	Green
Internode Direction	Straight	Straight	Straight	Straight

TABLE 4-continued

MORPHOLOGICAL TRAITS FOR THE 87DIA4 PHENOTYPE				
CHARACTERISTIC	87DIA4	2FACC	3AZA1	AQA3
Internode Length cm.	10.2	12.7	14.0	11.3
2. LEAF				
Color	Med Green	Med Green	Med Green	Med Green
Length cm.	68.0	71.1	66.2	67.9
Width cm.	10.0	8.7	7.9	8.3
Sheath Anthocyanin	Weak	Weak	Weak	Absent
Sheath Pubescence	Medium	Light	Medium	Medium
Marginal Waves	Few	Few	Few	Few
Longitudinal Creases	Absent	Absent	—	Few
3. TASSEL				
Attitude	Compact	Compact	—	Open
Length cm.	29.5	26.7	33.0	33.0
Spike Length cm.	19.5	19.1	24.4	23.1
Peduncle Length cm.	2.9	5.2	3.6	3.6
Branch Number	4.5	7.7	3.8	5.5
Anther Color	Red	Pink	Tan	Grn-Yellow
Glume Color	Green	Green	Green	Green
Glume Band	Absent	Absent	Absent	Absent
4. EAR				
Silk Color	Pink	Tan	Grn-Yellow	Grn-Yellow
Number Per Stalk	1.1	1.2	1.6	1.4
Position (attitude)	Upright	Upright	Pendant	Upright
Length cm.	15.6	13.9	16.4	16.3
Shape	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Diameter cm.	3.8	4.2	3.4	3.6
Weight gm.	99.1	116.3	89.8	93.1
Shank Length cm.	16.5	14.8	20.7	14.9
Husk Bract	Short	Short	Short	Short
Husk Cover cm.	3.4	6.6	1.9	3.2
Husk Opening	Tight	Intermediate	—	Intermediate
Husk Color Fresh	Green	Lt Green	Green	Lt Green
Husk Color Dry	Buff	Buff	Buff	Buff
Cob Diameter cm.	2.5	2.6	1.7	2.1
Cob Color	Red	Red	Red	Red
Shelling Percent	85.1	81.4	85.8	85.0
5. KERNEL				
Row Number	14.0	14.7	12.3	15.1
Number Per row	31.4	25.5	32.2	33.2
Row Direction	Curved	Curved	Curved	Curved
Type	Dent	Dent	Dent	Dent
Cap Color	Yellow	Yellow	Yellow	Lemon Yellow
Side Color	Yellow	Deep Yellow	Orange	Orange
Length (depth) mm.	10.2	10.7	9.2	9.6
Width mm.	8.1	8.1	7.3	7.1
Thickness	4.3	4.3	4.2	3.8
Weight of 1000K gm.	281.5	280.7	223.8	173.7
Endosperm Type	Normal	Normal	Normal	Normal
Endosperm Color	Yellow	Yellow	Yellow	Yellow

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

IV. ADDITIONAL INBRED CORN PLANTS

The inbred corn plant 171KI3 has been employed with the corn plant of the present invention in order to produce an exemplary hybrid. A description of the physiological and morphological characteristics of this corn plant is presented herein at Table 5. Additional information for this inbred corn plant is presented in co-pending U.S. patent application Ser. No. 08/795,403, filed Feb. 5, 1997, the disclosure of which application is specifically incorporated herein by reference.

TABLE 5

MORPHOLOGICAL TRAITS FOR THE 171KI3 PHENOTYPE				
CHARACTERISTIC	17IKI3	01CSI2	011BH2	3IIH6
1. STALK				
Diameter (width) cm.	2.2	2.4	2.1	2.3

TABLE 5-continued

MORPHOLOGICAL TRAITS FOR THE 171KI3 PHENOTYPE				
CHARACTERISTIC	171KI3	01CSI2	01IBH2	3IIH6
Anthocyanin	Absent	Absent	Absent	Absent
Nodes With Brace Roots	0.9	1.8	1.1	0.7
Brace Root Color	Green	Green	Green	—
Internode Direction	Straight	Straight	Straight	Straight
Internode Length cm.	15.9	12.8	14.4	13.1
2. LEAF				
Color	Med Green	—	Med Green	Med Green
Width cm.	9.7	8.9	8.9	8.6
Marginal Waves	Few	Few	Few	Few
3. TASSEL				
Length cm.	42.6	31.2	33.6	35.3
Spike Length cm.	22.9	23.2	23.1	25.2
Peduncle Length cm.	9.6	3.9	8.2	7.6
Branch Number	9.3	7.4	7.8	12.9
Anther Color	Purple	Grn-Yellow	Grn-Yellow	—
Glume Color	Purple	Green	Green	—
Glume Band	Present	Absent	Absent	Absent
4. EAR	Pink	Pink	—	Red
Silk Color	1.0	1.0	1.0	1.4
Number Per Stalk	Upright	—	—	Upright
Position (attitude)	14.6	16.0	14.6	15.6
Length cm.	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Shape	4.0	3.8	4.0	3.9
Diameter cm.	104.9	100.6	103.2	107.6
Weight gm.	10.3	14.1	10.1	9.6
Shank Length cm.	Short	Short	Short	Short
Husk Bract	6.4	2.5	4.4	3.7
Husk Cover cm.	Green	Green	Green	Green
Husk Color Fresh	Buff	Buff	Buff	Buff
Husk Color Dry	2.3	2.4	2.3	2.1
Cob Diameter cm.	Red	—	Red	Red
Cob Color	87.7	80.6	89.0	83.3
Shelling Percent				
5. KERNEL	14.6	14.8	16.3	15.3
Row Number	32.1	27.1	29.3	29.7
Number Per Row	Curved	Curved	Curved	Curved
Row Direction	Dent	—	Dent	—
Type	Yellow	Yellow	Yellow	Yellow
Cap Color	Deep Yellow	—	Orange	
Side Color	11.1	9.4	10.9	10.3
Length (depth) mm.	7.8	8.0	7.4	7.8
Width mm.	3.9	5.2	4.4	4.2
Thickness	269.0	252.4	233.0	247.8
Weight of 1000K gm.	Normal	Normal	Normal	Normal
Endosperm Type	Yellow	Yellow	Yellow	Yellow
Endosperm Color				

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

V. SINGLE GENE CONVERSIONS

When the term inbred corn plant is used in the context of the present invention, this also includes any single gene conversions of that inbred. The term single gene converted plant as used herein refers to those corn plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single gene transferred into the inbred via the backcrossing technique. Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the inbred. The term backcrossing as used herein refers to the repeated crossing of a hybrid progeny back to one of the parental corn plants for that inbred. The parental corn plant which contributes the gene for the desired characteristic is termed the nonrecurrent or donor parent. This terminology refers to the fact that the nonrecurrent parent is used one time in the backcross protocol and therefore does not recur. The parental corn plant to which the gene or genes from the nonrecurrent parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol (Poehlman & Sleper, 1994; Fehr, 1987). In a typical backcross protocol, the original inbred of interest (recurrent parent) is crossed to a second inbred (nonrecurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a corn plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the

converted plant, in addition to the single transferred gene from the nonrecurrent parent.

The selection of a suitable recurrent parent is an important step for a successful backcrossing procedure. The goal of a backcross protocol is to alter or substitute a single trait or characteristic in the original inbred. To accomplish this, a single gene of the recurrent inbred is modified or substituted with the desired gene from the nonrecurrent parent, while retaining essentially all of the rest of the desired genetic, and therefore the desired physiological and morphological, constitution of the original inbred. The choice of the particular nonrecurrent parent will depend on the purpose of the backcross, one of the major purposes is to add some commercially desirable, agronomically important trait to the plant. The exact backcrossing protocol will depend on the characteristic or trait being altered to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele may also be transferred. In this instance it may be necessary to introduce a test of the progeny to determine if the desired characteristic has been successfully transferred.

Many single gene traits have been identified that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques. Single gene traits may or may not be transgenic, examples of these traits include but are not limited to, male sterility, waxy starch, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, male fertility, enhanced nutritional quality, industrial usage, yield stability and yield enhancement. These genes are generally inherited through the nucleus. Some known exceptions to this are the genes for male sterility, some of which are inherited cytoplasmically, but still act as single gene traits. Several of these single gene traits are described in U.S. Ser. No. 07/113,561, filed Aug. 25, 1993, the disclosure of which is specifically hereby incorporated by reference.

Direct selection may ~~be~~ applied where the single gene acts as a dominant trait. An example might be the herbicide resistance trait. For this selection process, the progeny of the initial cross are sprayed with the herbicide prior to the backcrossing. The spraying eliminates any plants which do not have the desired herbicide resistance characteristic, and only those plants which have the herbicide resistance gene are used in the subsequent backcross. This process is then repeated for all additional backcross generations.

The waxy characteristic is an example of a recessive trait. In this example, the progeny resulting from the first backcross generation (BC1) must be grown and selfed. A test is then run on the selfed seed from the BC1 plant to determine which BC1 plants carried the recessive gene for the waxy trait. In other recessive traits, additional progeny testing, for example growing additional generations such as the BC1S1 may be required to determine which plants carry the recessive gene.

VI. ORIGIN AND BREEDING HISTORY OF AN EXEMPLARY SINGLE GENE CONVERTED PLANT

85DGD1 MLms is a single gene conversion of 85DGD1 to cytoplasmic male sterility. 85DGD1 MLms was derived using backcross methods. 85DGD1 (a proprietary inbred of DEKALB Genetics Corporation) was used as the recurrent parent and MLms, a germplasm source carrying ML cytoplasmic sterility, was used as the nonrecurrent parent. The breeding history of the single gene converted inbred 85DGD1 MLms can be summarized as follows:

Hawaii Nurseries Planting Date Apr. 2, 1992 Made up S-O: Female row 585 male row 500

Hawaii Nurseries Planting Date Jul. 15, 1992 S-O was grown and plants were backcrossed times 85DGD1 (rows 444' 443)

Hawaii Nurseries Planting Date Bulked seed of the BC1 was grown and Nov. 18, 1992 backcrossed times 85DGD1 (rows V3-27' V3-26)

Hawaii Nurseries Planting Date Apr. 2, 1993 Bulked seed of the BC2 was gown and backcrossed times 85DGD1 (rows 37' 36)

Hawaii Nurseries Planting Date Jul. 14, 1993 Bulked seed of the BC3 was grown and backcrossed times 85DGD1 (rows 99' 98)

Hawaii Nurseries Planting Date Bulked seed of BC4 was grown and backcrossed Oct. 28, 1993 times 85DGD1 (rows KS-63' KS-62)

Summer 1994 A single ear of the BC5 was grown and backcrossed times 85DGD1 (MC94-822' MC94-822-7)

Winter 1994 Bulked seed of the BC6 was grown and backcrossed times 85DGD1 (3Q-1' 3Q-2)

Summer 1995 Seed of the BC7 was bulked and named 85DGD1 MLms.

VII. TISSUE CULTURE AND IN VITRO REGENERATION OF CORN PLANTS

A further aspect of the invention relates to tissue culture of corn plants designated 87DIA4. As used herein, the term "tissue culture" indicates a composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant. Exemplary types of tissue cultures are protoplasts, calli, plant clumps, and plant cells that are intact in plants or parts of plants, such as embryos, pollen, flowers, kernels, ears, cobs, leaves, husks, stalks, roots, root tips, anthers, silk and the like. In a preferred embodiment, tissue culture is embryos, protoplast, meristematic cells, pollen, leaves or anthers. Means for preparing and maintaining plant tissue culture are well known in the art. By way of example, a tissue culture comprising organs such as tassels or anthers, has been used to produce regenerated plants. (See, U.S. patent applications Ser. No. 07/992,637, filed Dec. 18, 1992 and 07/995,938, filed Dec. 21, 1992, now issued as U.S. Pat. No. 5,322,789, issued Jun. 21, 1994, the disclosures of which are incorporated herein by reference).

VIII. TASSEL/ANTHER CULTURE

Tassels contain anthers which in turn enclose microspores. Microspores develop into pollen. For anther/microspore culture, if tassels are the plant composition., they are preferably selected at a stage when the microspores are uninucleate, that is, include only one, rather than 2 or 3 nuclei. Methods to determine the correct stage are well known to those killed in the art and include mitramycin fluorescent staining (Pace et al., 1987), trypan blue (preferred) and acetocarmine squashing. The mid-uninucleate microspore stage has been found to be the developmental stage most responsive to the subsequent methods disclosed to ultimately produce plants.

Although microspore-containing plant organs such as tassels can generally be pretreated at any cold temperature below about 25° C., a range of 4 to 25° C. is preferred, and a range of 8 to 14° C. is particularly preferred. Although other temperatures yield embryoids and regenerated plants, cold temperatures produce optimum response rates compared to pretreatment at temperatures outside the preferred range. Response rate is measured as either the number of embryoids or the number of regenerated plants per number of microspores initiated in culture.

Although not required, when tassels are employed as the plant organ, it is generally preferred to sterilize their surface.

Following surface sterilization of the tassels, for example, with a solution of calcium hypochloride, the anthers are removed from about 70 to 150 spikelets (small portions of the tassels) and placed in a preculture or pretreatment medium. Larger or smaller amounts can be used depending on the number of anthers.

When one elects to employ tassels directly, tassels are preferably pretreated at a cold temperature for a predefined time, preferably at 10° C. for about 4 days. After pretreatment of a whole tassel at a cold temperature, dissected anthers are further pretreated in an environment that diverts microspores from their developmental pathway. The function of the preculture medium is to switch the developmental program from one of pollen development that of embryoid/callus development. An embodiment of such an environment in the form of a preculture medium includes a sugar alcohol, for example mannitol or sorbitol, inositol or the like. An exemplary synergistic combination is the use of mannitol at a temperature of about 10° C. for a period ranging from about 10 to 14 days. In a preferred embodiment, 3 ml of 0.3 M mannitol combined with 50 mg/l of ascorbic acid, silver nitrate and colchicine is used for incubation of anthers at 10° C. for between 10 and 14 days. Another embodiment is to substitute sorbitol for mannitol. The colchicine produces chromosome doubling at this early stage. The chromosome doubling agent is preferably only present at the preculture stage.

It is believed that the mannitol or other similar carbon structure or environmental stress induces starvation and functions to force microspores to focus their energies on entering developmental stages. The cells are unable to use, for example, mannitol as a carbon source at this stage. It is believed that these treatments confuse the cells causing them to develop as embryoids and plants from microspores. Dramatic increases in development from these haploid cells, as high as 25 embryoids in 10^4 microspores, have resulted from using these methods.

In embodiments where microspores are obtained from anthers, microspores can be released from the anthers into an isolation medium following the mannitol preculture step. One method of release is by disruption of the anthers, for example, by chopping the anthers into pieces with a sharp instrument, such as a razor blade, scalpel or Waring blender. The resulting mixture of released microspores, anther fragments and isolation medium are then passed through a filter to separate microspores from anther wall fragments. An embodiment of a filter is a mesh, more specifically, a nylon mesh of about 112 mm pore size. The filtrate which results from filtering the microspore-containing solution is preferably relatively free of anther fragments, cell walls and other debris.

In a preferred embodiment, isolation of microspores is accomplished at a temperature below about 25° C. and, preferably at a temperature of less than about 15° C. Preferably, the isolation media, dispersing tool (e.g., razor blade) funnels, centrifuge tubes and dispersing container (e.g., petri dish) are all maintained at the reduced temperature during isolation. The use of a precooled dispersing tool to isolate maize microspores has been reported (Gaillard et al., 1991).

Where appropriate and desired, the anther filtrate is then washed several times in isolation medium. The purpose of the washing and centrifugation is to eliminate any toxic compounds which are contained in the non-microspore part of the filtrate and are created by the chopping process. The centrifugation is usually done at decreasing spin speeds, for example, 1000, 750, and finally 500 rpms.

The result of the foregoing steps is the preparation of a relatively pure tissue culture suspension of microspores that are relatively free of debris and anther remnants.

To isolate microspores, an isolation media is preferred. An isolation media is used to separate microspores from the anther walls while maintaining their viability and embryogenic potential. An illustrative embodiment of an isolation media includes a 6 percent sucrose or maltose solution combined with an antioxidant such as 50 mg/l of ascorbic acid, 0.1 mg/l biotin and 400 mg/l of proline, combined with 10 mg/l of nicotinic acid and 0.5 mg/l $AgNO_3$. In another embodiment, the biotin and proline are omitted.

An isolation media preferably has a higher antioxidant level where used to isolate microspores from a donor plant (a plant from which a plant composition containing a microspore is obtained) that is field grown in contrast to greenhouse grown. A preferred level of ascorbic acid in an isolation medium is from about 50 mg/l to about 125 mg/l and, more preferably from about 50 mg/l to about 100 mg/l.

One can find particular benefit in employing a support for the microspores during culturing and subculturing. Any support that maintains the cells near the surface can be used. The microspore suspension is layered onto a support, for example by pipetting. There are several types of supports which are suitable and are within the scope of the invention. An illustrative embodiment of a solid support is a TRANSWELL® culture dish. Another embodiment of a solid support for development of the microspores is a bilayer plate wherein liquid media is on top of a solid base. Other embodiments include a mesh or a millipore filter. Preferably, a solid support is a nylon mesh in the shape of a raft. A raft is defined as an approximately circular support material which is capable of floating slightly above the bottom of a tissue culture vessel, for example, a petri dish, of about a 60 or 100 mm size, although any other laboratory tissue culture vessel will suffice. In an illustrative embodiment, a raft is about 55 mm in diameter.

Culturing isolated microspores on a solid support, for example, on a 10 mm pore nylon raft floating on 2.2 ml of medium in a 60 mm petri dish, prevents microspores from sinking into the liquid medium and thus avoiding low oxygen tension. These types of cell supports enable the serial transfer of the nylon raft with its associated microspore/embryoids ultimately to full strength medium containing activated charcoal and solidified with, for example, GELRITE™ (solidifying agent). The charcoal is believed to absorb toxic wastes and intermediaries. The solid medium allows embryoids to mature.

The liquid medium passes through the mesh while the microspores are retained and supported at the medium-air interface. The surface tension of the liquid medium in the petri dish causes the raft to float. The liquid is able to pass through the mesh: consequently, the microspores stay on top. The mesh remains on top of the total volume of liquid medium. An advantage of the raft is to permit diffusion of nutrients to the microspores. Use of a raft also permits transfer of the microspores from dish to dish during subsequent subculture with minimal loss, disruption or disturbance of the induced embryoids that are developing. The rafts represent an advantage over the multi-welled TRANSWELL® plates, which are commercially available from COSTAR, in that the commercial plates are expensive. Another disadvantage of these plates is that to achieve the serial transfer of microspores to subsequent media, the membrane support with cells must be peeled off the insert in the wells. This procedure does not produce as good a yield nor as efficient transfers, as when a mesh is used as a vehicle for cell transfer.

The culture vessels can be further defined as either (1) a bilayer 60 mm petri plate wherein the bottom 2 ml of medium are solidified with 0.7 percent agarose, overlaid with 1 mm of liquid containing the microspores; (2) a nylon mesh raft wherein a wafer of nylon is floated on 1.2 ml of medium and 1 ml of isolated microspores is pipetted on top; or (3) TRANSWELL® plates wherein isolated microspores are pipetted onto membrane inserts which support the microspores at the surface of 2 ml of medium.

After the microspores have been isolated, they are cultured in a low strength anther culture medium until about the 50 cell stage when they are subcultured onto an embryoid/callus maturation medium. Medium is defined at this stage as any combination of nutrients that permit the microspores to develop into embryoids or callus. Many examples of suitable embryoid/callus promoting media are well known to those skilled in the art. These media will typically comprise mineral salts, a carbon source, vitamins, growth regulations. A solidifying agent is optional. A preferred embodiment of such a media is referred to by the inventor as the "D medium" which typically includes 6N1 salts, $AgNO_3$ and sucrose or maltose.

In an illustrative embodiment, 1 ml of isolated microspores are pipetted onto a 10 mm nylon raft and the raft is floated on 1.2 ml of medium "D", containing sucrose or, preferably maltose. Both calli and embryoids can develop. Calli are undifferentiated aggregates of cells. Type I is a relatively compact, organized and slow growing callus. Type II is a soft, friable and fast-growing one. Embryoids are aggregates exhibiting some embryo-like structures. The embryoids are preferred for subsequent steps to regenerating plants. Culture medium "D" is an embodiment of medium that follows the isolation medium and replaces it. Medium "D" promotes growth to an embryoid/callus. This medium comprises 6N1 salts at ⅛ the strength of a basic stock solution, (major components) and minor components, plus 12 percent sucrose or, preferably 12 percent maltose, 0.1 mg/l B1, 0.5 mg/l nicotinic acid, 400 mg/l proline and 0.5 mg/l silver nitrate. Silver nitrate is believed to act as an inhibitor to the action of ethylene. Multi-cellular structures of approximately 50 cells each generally arise during a period of 12 days to 3 weeks. Serial transfer after a two week incubation period is preferred.

After the petri dish has been incubated for an appropriate period of time, preferably two weeks, in the dark at a predefined temperature, a raft bearing the dividing microspores is transferred serially to solid based media which promotes embryo maturation. In an illustrative embodiment, the incubation temperature is 30° C. and the mesh raft supporting the embryoids is transferred to a 100 mm petri dish containing the 6N1-TGR-4P medium, an "anther culture medium." This medium contains 6N1 salts, supplemented with 0.1 mg/l TIBA, 12 percent sugar (sucrose, maltose or a combination thereof), 0.5 percent activated charcoal, 400 mg/l proline, 0.5 mg/l B, 0.5 mg/l nicotinic acid, and 0.2 percent GELRITE™ (solidifying agent) and is capable of promoting the maturation of the embryoids. Higher quality embryoids, that is, embryoids which exhibit more organized development, such as better shoot meristem formation without precocious germination were typically obtained with the transfer to full strength medium compared to those resulting from continuous culture using only, for example, the isolated microspore culture (IMC) Medium "D." The maturation process permits the pollen embryoids to develop further in route toward the eventual regeneration of plants. Serial transfer occurs to full strength solidified 6N1 medium using either the nylon raft, the TRANSWELL® membrane or bilayer plates, each one requiring the movement of developing embryoids to permit further development into physiologically more mature structures.

In an especially preferred embodiment, microspores are isolated in an isolation media comprising about 6 percent maltose, cultured for about two weeks in an embryoid/calli induction medium comprising about 12 percent maltose and then transferred to a solid medium comprising about 12 percent sucrose.

At the point of transfer of the raft after about two weeks incubation, embryoids exist on a nylon support. The purpose of transferring the raft with the embryoids to a solidified medium after the incubation is to facilitate embryo maturation. Mature embryoids at this point are selected by visual inspection indicated by zygotic embryo-like dimensions and structures and are transferred to the shoot initiation medium. It is preferred that shoots develop before roots, or that shoots and roots develop concurrently. If roots develop before shoots, plant regeneration can be impaired. To produce solidified media, the bottom of a petri dish of approximately 100 mm is covered with about 30 ml of 0.2 percent GELRITE™ (solidifying agent) solidified medium. A sequence of regeneration media are used for whole plant formation from the embryoids.

During the regeneration process, individual embryoids are induced to form plantlets. The number of different media in the sequence can vary depending on the specific protocol used. Finally, a rooting medium is used as a prelude to transplanting to soil. When plantlets reach a height of about 5 cm, they are then transferred to pots for further growth into flowering plants in a greenhouse by methods well known to those skilled in the art.

Plants have been produced from isolated microspore cultures by methods disclosed herein, including self-pollinated plants. The rate of embryoid induction was much higher with the synergistic preculture treatment consisting of a combination of stress factors, including a carbon source which can be capable of inducing starvation, a cold temperature and colchicine, than has previously been reported. An illustrative embodiment of the synergistic combination of treatments leading to the dramatically improved response rate compared to prior methods, is a temperature of about 10° C., mannitol as a carbon source, and 0.05 percent colchicine.

The inclusion of ascorbic acid, an anti-oxidant, in the isolation medium is preferred for maintaining good microspore viability. However, there seems to be no advantage to including mineral salts in the isolation medium. The osmotic potential of the isolation medium was maintained optimally with about 6 percent sucrose, although a range of 2 percent to 12 percent is within the scope of this invention.

In an embodiment of the embryoid/callus organizing media, mineral salts concentration in IMC Culture Media "D" is (⅛x), the concentration which is used also in anther culture medium. The 6N1 salts major components have been modified to remove ammonium nitrogen. Osmotic potential in the culture medium is maintained with about 12 percent sucrose and about 400 mg/l proline. Silver nitrate (0.5 mg/l) was included in the medium to modify ethylene activity. The preculture media is further characterized by having a pH of about 5.7 to 6.0. Silver nitrate and vitamins do not appear to be crucial to this medium but do improve the efficiency of the response.

Whole anther cultures can also be used in the production of monocotyledonous plants from a plant culture system. There are some basic similarities of anther culture methods

and microspore culture methods with regard to the media used. A difference from isolated microspore cultures is that undisrupted anthers are cultured, so that a support, eg., a nylon mesh support, is not needed. The first step in developing the anther cultures is to incubate tassels at a cold temperature. A cold temperature is defined as less than about 25° C. More specifically, the incubation of the tassels is preferably performed at about 10° C. A range of 8 to 14° C. is also within the scope of the invention. The anthers are then dissected from the tassels, preferably after surface sterilization using forceps, and placed on solidified medium. An example of such a medium is designated by the inventors as 6N1-TGR-P4.

The anthers are then treated with environmental conditions that area combinations of stresses that are capable of diverting microspores from gametogenesis to embryogenesis. It is believed that the stress effect of sugar alcohols in the preculture medium, for example, mannitol, is produced by inducing starvation at the predefined temperature. In one embodiment, the incubation pretreatment is for about 14 days at 10° C. It was found that treating the anthers in addition with a carbon structure, an illustrative embodiment being a sugar alcohol, preferably, mannitol, produces dramatically higher anther culture response rates as measured by the number of eventually regenerated plants, than by treatment with either cold treatment or mannitol alone. These results are particularly surprising in light of teachings that cold is better than mannitol for these purposes, and that warmer temperatures interact with mannitol better.

To incubate the anthers, they are floated on a preculture medium which diverts the microspores from gametogenesis, preferably on a mannitol carbon structure,, more specifically, 0.3 M of mannitol plus 50 mg/l of ascorbic acid. 3 ml is about the total amount in a dish, for example, a tissue culture dish, more specifically, a 60 mm petri dish. Anthers are isolated from about 120 spikelets for one dish yields about 360 anthers.

Chromosome doubling agents can be used in the preculture media for anther cultures. Several techniques for doubling chromosome number (Jensen, 1974; Wan et al., 1989) have been described. Colchicine is one of the doubling agents. However, developmental abnormalities arising from in vitro cloning are further enhanced by colchicine treatments, and previous reports indicated that colchicine is toxic to microspores. The addition of colchicine in increasing concentrations during mannitol pretreatment prior to anther culture and microspore culture has achieved improved percentages.

An illustrative embodiment of the combination of a chromosome doubling agent and preculture medium is one which contains colchicine. In a specific embodiment, the colchicine level is preferably about 0.05 percent. The anthers remain in the mannitol preculture medium with the additives for about 10 days at 10° C. Anthers are then placed on maturation media, for example, that designated 6N1-TGR-P4, for 3 to 6 weeks to induce embryoids. If the plants are to be regenerated from the embryoids, shoot regeneration medium is employed, as in the isolated microspore procedure described in the previous sections. Other regeneration media can be used sequentially to complete regeneration of whole plants.

The anthers are then exposed to embryoid/callus promoting medium, for example, that designated 6N1-TGR-P4 to obtain callus or embryoids. The embryoids are recognized by identification visually of embryonic-like structures. At this stage, the embryoids are transferred serially to a series of regeneration media. In an illustrative embodiment, the shoot initiation medium comprises BAP (6-benzyl-aminopurine) and NAA (naphthalene acetic acid). Regeneration protocols for isolated microspore cultures and anther cultures are similar.

IX. OTHER CULTURES AND REGENERATION

The present invention contemplates a corn plant regenerated from a tissue culture of an inbred (e.g., 87DIA4) or hybrid plant (e.g., 4033843) of the present invention. As is well known in the art, tissue culture of corn can be used for the in vitro regeneration of a corn plant. By way of example, a process of tissue culturing and regeneration of corn is described in European Patent Application, publication 160, 390, the disclosure of which is incorporated by reference. Corn tissue culture procedures are also described in Green & Rhodes (1982) and Duncan et al., (1985). The study by Duncan et al. (1985) indicates that 97 percent of cultured plants produced calli capable of regenerating plants. Subsequent studies have shown that both inbreds and hybrids produced 91 percent regenerable calli that produced plants.

Other studies indicate that non-traditional tissues are capable of producing somatic embryogenesis and plant regeneration. See, e.g., Songstad et al. (1988); Rao et al. (1986); and Conger et al. (1987), the disclosures of which are incorporated herein by reference. Regenerable cultures may be initiated from immature embryos as described in PCT publication WO 95/06128, the disclosure of which is incorporated herein by reference.

Briefly, by way of example, to regenerate a plant of this invention, cells are selected following growth in culture. Where employed, cultured cells are preferably grown either on solid supports or in the form of liquid suspensions as set forth above. In either instance, nutrients are provided to the cells in the form of media, and environmental conditions are controlled. There are many types of tissue culture media comprising amino acids, salts, sugars, hormones and vitamins. Most of the media employed to regenerate inbred and hybrid plants have some similar components, the media differ in the composition and proportions of their ingredients depending on the particular application envisioned. For example, various cell types usually grow in more than one type of media, but exhibit different growth rates and different morphologies, depending on the growth media. In some media, cells survive but do not divide. Various types of media suitable for culture of plant cells have been previously described and discussed above.

An exemplary embodiment for culturing recipient corn cells in suspension cultures includes using embryogenic cells in Type II (Armstrong & Green, 1985; (Gordon-Kamm et al., 1990) callus, selecting for small (10 to 30 m) isodiametric, cytoplasmically dense cells, growing the cells in suspension cultures with hormone containing media, subculturing into a progression of media to facilitate development of shoots and roots, and finally, hardening the plant and readying it metabolically for growth in soil.

Meristematic cells (i.e., plant cells capable of continual cell division and characterized by an undifferentiated cytological appearance, normally found at growing points or tissues in plants such as root tips, stem apices, lateral buds, etc.) can be cultured.

Embryogenic calli are produced essentially as described in PCT Publication WO 95/06128. Specifically, inbred plants or plants from hybrids produced from crossing an inbred of the present invention with another inbred are grown to flowering in a greenhouse. Explants from at least one of the following F_1 tissues: the immature tassel tissue, intercalary meristems and leaf bases, apical meristems, immature ears and immature embryos are placed in an

initiation medium which contain MS salts, supplemented with thiamine, agar, and sucrose. Cultures are incubated in the dark at about 23° C. All culture manipulations and selections are performed with the aid of a dissecting microscope.

After about 5 to 7 days, cellular outgrowths are observed from the surface of the explants. After about 7 to 21 days, the outgrowths are subcultured by placing them into fresh medium of the same composition. Some of the intact immature embryo explants are placed on fresh medium. Several subcultures later (after about 2 to 3 months) enough material is present from explants for subdivision of these embryogenic calli into two or more pieces.

Callus pieces from different explants are not mixed. After further growth and subculture (about 6 months after embryogenic callus initiation), there are usually between 1 and 100 pieces derived ultimately from each selected explant. During this time of culture expansion, a characteristic embryogenic culture morphology develops as a result of careful selection at each subculture. Any organized structures resembling roots or root primordia are discarded. Material known from experience to lack the capacity for sustained growth is also discarded (translucent, watery, embryogenic structures). Structures with a firm consistency resembling at least in part the scutelum of the in vivo embryo are selected.

The callus is maintained on agar-solidified MS or N6-type media. A preferred hormone is 2,4-D. A second preferred hormone is dicamba. Visual selection of embryo-like structures is done to obtain subcultures. Transfer of material other than that displaying embryogenic morphology results in loss of the ability to recover whole plants from the callus.

Cell suspensions are prepared from the calli by selecting cell populations that appear homogeneous macroscopically. A portion of the friable, rapidly growing embryogenic calli is inoculated into MS or N6 Medium containing 2,4-D or dicamba. The calli in medium are incubated at about 27° C. on a gyrotary shaker in the dark or in the presence of low light. The resultant suspension culture is transferred about once every three to seven days, preferably every three to four days, by taking about 5 to 10 ml of the culture and introducing this inoculum into fresh medium of the composition listed above.

For regeneration, embryos which appear on the callus surface are selected and regenerated into whole plants by transferring the embryogenic structure, into a sequence of solidified media which include decreasing concentrations of 2,4-D or other auxins. Other hormones which can be used in culture media include dicamba, NAA, ABA, BAP, and 2-NCA. The reduction is relative to the concentration used in culture maintenance media. Plantlets are regenerated from these embryos by transfer to a hormone-free medium, subsequently transferred to soil, and grown to maturity.

Progeny are produced by taking pollen and selfing, backcrossing or sibling regenerated plants by methods well known to those skilled in the arts. Seeds are collected from the regenerated plants.

X. PROCESSES OF PREPARING CORN PLANTS AND THE CORN PLANTS PRODUCED BY SUCH CROSSES

The present invention also provides a process of preparing a novel corn plant and a corn plant produced by such a process. In accordance with such a process, a first parent corn plant is crossed with a second parent corn plant wherein at least one of the first and second corn plants is the inbred corn plant 87DIA4. In one embodiment, a corn plant prepared by such a process is a first generation F_1 hybrid corn plant prepared by a process wherein both the first and second parent corn plants are inbred corn plants.

Corn plants (*Zea mays L.*) can be crossed by either natural or mechanical techniques. Natural pollination occurs in corn when wind blows pollen from the tassels to the silks that protrude from the tops of the incipient ears. Mechanical pollination can be effected either by controlling the types of pollen that can blow onto the silks or by pollinating by hand.

In a preferred embodiment, crossing comprises the steps of:

(a) planting in pollinating proximity seeds of a first and a second parent corn plant, and preferably, seeds of a first inbred corn plant and a second, distinct inbred corn plant;

(b) cultivating or growing the seeds of the first and second parent corn plants into plants that bear flowers;

(c) emasculating flowers of either the first or second parent corn plant, i.e., treating the flowers so as to prevent pollen production, in order to produce an emasculated parent corn plant;

(d) allowing natural cross-pollination to occur between the first and second parent corn plant;

(e) harvesting seeds produced on the emasculated parent corn plant; and, where desired,

(f) growing the harvested seed into a corn plant, or preferably, a hybrid corn plant.

Parental plants are planted in pollinating proximity to each other by planting the parental plants in alternating rows, in blocks or in any other convenient planting pattern. Plants of both parental parents are cultivated and allowed to grow until the time of flowering. Advantageously, during this growth stage, plants are in general treated with fertilizer and, or other agricultural chemicals as considered appropriate by the grower.

At the time of flowering, in the event that plant 87DIA4, is employed as the male parent, the tassels of the other parental plant are removed from all plants employed as the female parental plant. The detasseling can be achieved manually but also can be done by machine, if desired.

The plants are then allowed to continue to grow and natural cross-pollination occurs as a result of the action of wind, which is normal in the pollination of grasses, including corn. As a result of the emasculation of the female parent plant, all the pollen from the male parent plant 87DIA4 is available for pollination because tassels, and thereby pollen bearing flowering parts, have been previously removed from all plants of the inbred plant being used as the female in the hybridization. Of course, during this hybridization procedure, the parental varieties are grown such that they are isolated from other corn fields to minimize or prevent my accidental contamination of pollen from foreign sources. These isolation techniques are well within the skill of those skilled in this art.

Both parental inbred plants of corn may be allowed to continue to grow until maturity or the male rows may be destroyed after flowering is complete. Only the ears from the female inbred parental plants are harvested to obtain seeds of a novel F_1 hybrid. The novel F_1 hybrid seed produced can then be planted in a subsequent growing season with the desirable characteristics in terms of F_1 hybrid corn plants providing improved grain yields and the other desirable characteristics disclosed herein, being achieved.

Alternatively, in another embodiment, both first and second parent corn plants can come from the same inbred corn plant, i.e., from the inbred designated 87DIA4. Thus, any corn plant produced using a process of the present invention and inbred corn plant 87DIA4, is contemplated by this invention. As used herein, crossing can mean selfing,

backcrossing, crossing to another or the same inbred, crossing to populations, and the like. All corn plants produced using the present inbred corn plant 87DIA4 as a parent are within the scope of this invention.

The utility of the inbred plant 87DIA4 also extends to crosses with other species. Commonly, suitable species will be of the family Graminaceae, and especially of the genera Zea, Tripsacum, Coix, Schlerachne, Polytoca, Chionachne, and Trilobachne, of the tribe Maydeae. Of these, Zea and Tripsacum, are most preferred. Potentially suitable for crosses with 87DIA4 can be the various varieties of grain sorghum, *Sorghum bicolor* (L.) Moench.

A. F_1 HYBRID CORN PLANT AND SEED PRODUCTION

Where the inbred corn plant 87DIA4 is crossed with another, different, corn inbred, a first generation (F_1) corn hybrid plant is produced. Both a F_1 hybrid corn plant and a seed of that F_1 hybrid corn plant are contemplated as aspects of the present invention.

Inbred 87DIA4 has been used to prepare an F_1 hybrid corn plant, designated 4033843.

The goal of a process of producing an F_1 hybrid is to manipulate the genetic complement of corn to generate new combinations of genes which interact to yield new, or improved traits (phenotypic characteristics). A process of producing an F_1 hybrid typically begins with the production of one or more inbred plants. Those plants are produced by repeated crossing of ancestrally related corn plants to try and concentrate certain genes within the inbred plants. The production of inbred 87DIA4 has been set forth hereinbefore.

Corn has a diploid phase which means two conditions of a gene (two alleles) occupy each locus (position on a chromosome). If the alleles are the same at a locus, there is said to be homozygosity. If they are different, there is said to be heterozygosity. In a completely inbred plant, all loci are homozygous. Because many loci when homozygous are deleterious to the plant, in particular leading to reduced vigor, less kernels, weak and/or poor growth, production of inbred plants is an unpredictable and arduous process. Under some conditions, heterozygous advantage at some loci effectively bars perpetuation of homozygosity.

Inbreeding requires coddling and sophisticated manipulation by human breeders. Even in the extremely unlikely event inbreeding rather than crossbreeding occurred in natural corn, achievement of complete inbreeding cannot be expected in nature due to well known deleterious effects of homozygosity and the large number of generations the plant would have to breed in isolation. The reason for the breeder to create inbred plants is to have a known reservoir of genes whose gametic transmission is at least somewhat predictable.

The development of inbred plants generally requires at least about 5 to 7 generations of selfing. Inbred plants are then cross-bred in an attempt to develop improved F_1 hybrids. Hybrids are then screened and evaluated in small scale field trials. Typically, about 10 to 15 phenotypic traits, selected for their potential commercial value, are measured. A selection index of the most commercially important traits is used to help evaluate hybrids. FACT, an acronym for Field Analysis Comparison Trial (strip trials), is an on-farm testing program employed by DEKALB Plant Genetics to perform the final evaluation of the commercial potential of a product.

During the next several years, a progressive elimination of hybrids occurs based on more detailed evaluation of their phenotype. Eventually, strip trials (FACT) are conducted to formally compare the experimental hybrids being developed with other hybrids, some of which were previously developed and generally are commercially successful. That is, comparisons of experimental hybrids are made to competitive hybrids to determine if there was any advantage to further commercial development of the experimental hybrids. Examples of such comparisons are presented in Section B, hereinbelow.

When the inbred parental plant 87DIA4 is crossed with another inbred plant to yield a hybrid (such as the hybrid 4033843), the original inbred can serve as either the maternal or paternal plant. For many crosses, the outcome is the same regardless of the assigned sex of the parental plants.

However, there is often one of the parental plants that is preferred as the maternal plant because of increased seed yield and production characteristics. Some plants produce tighter ear husks leading to more loss, for example due to rot. There can be delays in silk formation which deleteriously affect timing of the reproductive cycle for a pair of parental inbreds. Seed coat characteristics can be preferable in one plant. Pollen can be shed better by one plant. Other variables can also affect preferred sexual assignment of a particular cross.

B. F_1 HYBRID COMPARISONS

As mentioned in Section A, hybrids are progressively eliminated following detailed evaluations of their phenotype, including formal comparisons with other commercially successful hybrids. Strip trials are used to compare the phenotypes of hybrids grown in as many environments as possible. They are performed in many environments to assess overall performance of the new hybrids and to select optimum growing conditions. Because the corn is grown in close proximity, environmental factors that affect gene expression, such as moisture, temperature, sunlight and pests, are minimized. For a decision to be made that a hybrid is worth making commercially available, it is not necessary that the hybrid be better than all other hybrids. Rather, significant improvements must be shown in at least some traits that would create improvements in some niches.

Examples of such comparative data are set forth hereinbelow in Table 6, which presents a comparison of performance data for the hybrid 4033843, a hybrid made with 87DIA4 as one parent, versus a selected hybrid of commercial value (DK442).

All the data in Table 6 represents results across years and locations for research and/or strip trials. The "NTEST" represents the number of paired observations in designated tests at locations around the United States.

TABLE 6

COMPARATIVE DATA FOR 4033843

HYBRID	NTEST	SI %C	YLD BU	MST PTS	STL %	RTL %	DRP %	FLSTD %M	SV RAT

TABLE 6-continued

COMPARATIVE DATA FOR 4033843

4033843	R 93	110.3	156.3	19.9	5.2	1.4	0.1	101.0	4.1
DK442		99.0	147.7	19.6	7.1	5.2	0.1	100.9	4.1
DEV		11.3**	8.6**	0.1	-1.9**	-3.8**	0.0	0.2	-0.1

HYBRID	NTEST	ELSTD % M	PHT INCH	EHT INCH	BAR %	SG RAT	TST LBS	FGDU	ESTR DAYS
4033843	R 93	104.4	89.8	40.9		5.0	54.2	1214.0	94.0
DK442		102.9	90.2	43.9		3.1	53.4	1251.0	93.9
DEV		1.5+	-0.4	-3.0**		1.8**	0.8**	-36.9**	0.1

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
LEGEND ABBREVIATIONS:
HYBD = Hybrid
TEST = Research/FACT
SI % C = Selection Index (percent of check)
YLD BU = Yield (bushels/acre)
MST PTS = Moisture
STL % = Stalk Lodging (percent)
RTL % = Root Lodging (percent)
DRP % = Dropped Ears (percent)
FLSTD % M = Final Stand (percent of test mean)
SV RAT = Seedling Vigor Rating
ELSTD % M = Early Stand (percent of test mean)
PHT INCH = Plant Height (inches)
EHT INCH = Ear Height (inches)
BAR % = Barren Plants (percent)
SG RAT = Staygreen Rating
TST LBS = Test Weight (pounds)
FGDU = GDUs to Shed
ESTR DAYS = Estimated Relative Maturity (days)

As can be seen in Table 6, the hybrid 4033843 has significantly higher yield with comparable moisture content when compared to a successful commercial hybrid. Significant differences are also shown in Table 6 for many other traits.

C. PHYSICAL DESCRIPTION OF F_1 HYBRIDS

The present invention also provides F_1 hybrid corn plants derived from the corn plant 87DIA4. Physical characteristics of exemplary hybrids are set forth in Table 7, which concerns 4033843, which has 87DIA4 as one inbred parent. An explanation of terms used in Table 7 can be found in the Definitions, set forth herein above.

TABLE 7

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996

	CHARACTERISTIC	VALUE
1.	STALK	
	Diameter (width) cm	2.6
	Anthocyanin	Absent
	Nodes with Brace Roots	1.5
	Brace Root Color	Red
	Internode Direction	Straight
	Internode Length cm.	16.0
2.	LEAF	
	Color	Med Green
	Length cm.	79.9
	Width cm.	10.7
	Sheath Anthocyanin	Absent
	Sheath Pubescence	Medium
	Marginal Waves	Medium
	Longitudinal Creases	Few
3.	TASSEL	
	Attitude	Compact
	Length cm.	48.1
	Spike Length cm.	27.4
	Peduncle Length cm.	12.1
	Branch Number	7.5
	Anther Color	Red
	Glume Color	Purple
	Glume Band	Absent
4.	EAR	
	Silk Color	Tan
	Number Per Stalk	1.1
	Position (attitude)	Upright
	Length cm.	20.7
	Shape	Semi-conical
	Diameter cm.	4.7
	Weight gm.	222.8
	Shank Length cm.	18.7
	Husk Bract	Short
	Husk Opening	Open
	Husk Color Fresh	Green
	Husk Color Dry	Buff
	Cob Diameter cm.	2.4
	Cob Color	Red
	Shelling Percent	88.6
5.	KERNEL	
	Row Number	15.4
	Number Per row	42.6
	Row Direction	Straight
	Type	Dent

TABLE 7-continued

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996	
CHARACTERISTIC	VALUE
Cap Color	Yellow
Side Color	Deep Yellow
Length (depth) mm.	12.0
Width mm.	7.9
Thickness	4.1
Weight of 1000K gm.	307.0
Endosperm Type	Normal
Endosperm Color	Yellow

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

XI. GENETIC COMPLEMENTS

In another aspect, the present invention provides a genetic complement of a plant of this invention. In one embodiment, therefore, the present invention contemplates an inbred genetic complement of the inbred corn plant designated 87DIA4. In another embodiment, the present invention contemplates a hybrid genetic complement formed by the combination of a haploid genetic complement from 87DIA4 and another haploid genetic complement. Means for determining a genetic complement are well-known in the art.

As used herein, the phrase "genetic complement" means an aggregate of nucleotide sequences, the expression of which sequences defines the phenotype of a corn plant or a cell or tissue of that plant. By way of example, a corn plant is genotyped to determine the array of the inherited markers it possesses. Markers are alleles at a single locus. They are preferably inherited in codominant fashion so that the presence of both alleles at a diploid locus is readily detectable, and they are free of environmental variation, i.e., their heritability is 1. This genotyping is preferably performed on at least one generation of the descendant plant for which the numerical value of the quantitative trait or traits of interest are also determined. The array of single locus genotypes is expressed as a profile of marker alleles, two at each locus. The marker allelic composition of each locus can be either homozygous or heterozygous. Homozygosity is a condition where both alleles at a locus are characterized by the same nucleotide sequence. Heterozygosity refers to different conditions of the gene at a locus. Markers that are used for purposes of this invention include restriction fragment length polymorphisms (RFLPs) and isozymes.

A plant genetic complement can be defined by genetic marker profiles that can be considered "fingerprints" of a genetic complement. For purposes of this invention, markers are preferably distributed evenly throughout the genome to increase the likelihood they will be near a quantitative trait loci (QTL) of interest (e.g., in tomatoes, Helentjaris et al., U.S. Pat. No. 5,385,835, Nienhuis et al., 1987). These profiles are partial projections of a sample of genes. One of the uses of markers in general is to exclude, or alternatively include, potential parents as contributing to offspring.

Phenotypic traits characteristic of the expression of a genetic complement of this invention are distinguishable by electrophoretic separation of DNA sequences cleaved by various restriction endonucleases. Those traits (genetic markers) are termed RFLPs (restriction fragment length polymorphisms).

Restriction fragment length polymorphisms (RFLPs) are genetic differences detectable by DNA fragment lengths, typically revealed by agarose gel electrophoresis, after restriction endonuclease digestion of DNA. There are large numbers of restriction endonucleases available, characterized by their nucleotide cleavage sites and their source, e.g., Eco RI. Variations in RFLPs result from nucleotide base pair differences which alter the cleavage sites of the restriction endonucleases, yielding different sized fragments.

Means for performing RFLP analyses are well known in the art. Restriction fragment length polymorphism analyses reported herein were conducted by Linkage Genetics. This service is available to the public on a contractual basis. Probes were prepared to the fragment sequences, these probes being complementary to the sequences thereby being capable of hybridizing to them under appropriate conditions well known to those skilled in the art. These probes were labeled with radioactive isotopes or fluorescent dyes for ease of detection. After the fragments were separated by size, they were identified by the probes. Hybridization with a unique cloned sequence permits the identification of a specific chromosomal region (locus). Because all alleles at a locus are detectable, RFLPs are codominant alleles, thereby satisfying a criteria for a genetic marker. They differ from some other types of markers, e.g., from isozymes, in that they reflect the primary DNA sequence, they are not products of transcription or translation. Furthermore, different RFLP genetic marker profiles result from different arrays of restriction endonucleases.

The RFLP genetic marker profile of each of the parental inbreds and exemplary resultant hybrids were determined. Because an inbred is essentially homozygous at all relevant loci, an inbred should, in almost all cases, have only one allele at each locus. In contrast, a diploid genetic marker profile of a hybrid should be the sum of those parents, e.g., if one inbred parent had the allele A at a particular locus, and the other inbred parent had B, the hybrid is AB by inference. Subsequent generations of progeny produced by selection and breeding are anticipated to be of genotype A, B, or AB for that locus position. When the F1 plant is used to produce an inbred, the locus should be either A or B for that position. Surprisingly, it has been observed that in certain instances, novel RFLP genotypes arise during the breeding process. For example, a genotype of C is observed at a particular locus position from the cross of parental inbreds with A and B at that locus. Such a novel RFLP genotype is observed for the 87DIA4, at least, for the RFLP markers M5213S and M8B2369S, as shown in Table 8. These novel RFLP markers further define the 87DIA4 inbred from the parental inbreds from which it was derived. An RFLP genetic marker profile of 87DIA4 is presented in Table 8.

TABLE 8

RFLP PROFILE OF 87DIA4				
PROBE/ENZYME	87DIA4	2FACC	3AZA1	AQA3
M0264H	D	G	G	D
M0306H	A	A	—	A
M0445E	C	B	B	C
M1120S	F	—	D	F
M1234H	D	D	E	E
M1236H	A	A	—	A
M1238H	A	A	F	K
M1401E	C	C	C	A
M1406H	A	—	B	B
M1447H	B	B	E	B
M1B725E	B	B	C	C
M2239H	A	A	C	C
M2297H	A	A	E	A

TABLE 8-continued

RFLP PROFILE OF 87DIA4

PROBE/ENZYME	87DIA4	2FACC	3AZA1	AQA3
M2298E	C	B	C	C
M2402H	E	E	E	E
M3212S	A	A	B	A
M3247E	D	B	D	D
M3257S	B	B	B	B
M3296H	D	A	D	D
M3432H	A	I	A	A
M3446S	C	B	C	C
M3457E	E	E	E	E
M4386H	B	B	A	A
M4396H	H	H	F	F
M4444H	B	B	A	A
M4UMC19H	A	A	A	A
M4UMC31S	D	A	B	D
M5213S	B	A	B	A
M5295E	C	D	C	C
M5408H	A	A	A	A
M5579S	B	B	B	B
M5UMC95H	A	A	B	B
M6223E	C	C	C	C
M6252H	D	—	D	E
M6280H	E	E	A	A
M6373E	A	E	A	A
M7263E	A	C	A	A
M7391H	C	C	A	A
M7392S	C	C	B	C
M7455H	A	A	C	C
M8110S	C	C	C	C
M8114E	B	B	E	E
M8268H	B	B	B	B
M8585H	A	A	A	A
M8B2369S	B	D	B	D
M8UMC48E	C	C	C	C
M9209E	C	C	A	A
M9266S	A	A	C	C
M9B713S	A	A	B	B
M2UMC34H	D	D	D	—
M6UMC85H	A	A	A	—
M9UMC94H	E	E	B	—
M3UM121X	C	C	C	—
M0UMC130	C	H	—	—

*Probes used to detect RFLPs are from Linkage Genetics, 1515 West 2200 South, Suite C, Salt Lake City, Utah 84119.

Another aspect of this invention is a plant genetic complement characterized by a genetic isozyme typing profile. Isozymes are forms of proteins that are distinguishable, for example, on starch gel electrophoresis, usually by charge and/or molecular weight. The techniques and nomenclature for isozyme analysis are described in, Stuber et al. (1988), which is incorporated by reference.

A standard set of loci can be used as a reference set. Comparative analysis of these loci is used to compare the purity of hybrid seeds, to assess the increased variability in hybrids compared to inbreds, and to determine the identity of seeds, plants, and plant parts. In this respect, an isozyme reference set can be used to develop genotypic "fingerprints."

Table 9 lists the identifying numbers of the alleles at isozyme loci types, and represents the exemplary genetic isozyme typing profile for 87DIA4.

TABLE 9

ISOZYME PROFILE OF 87DIA4

LOCUS	ISOZYME ALLELE			
	87DIA4	2FACC	3AZA1	AQA3
Acph1	2	2	4	4
Adh1	4	4	4	4
Cat3	9	9	9	9
Got1	4	4	4	4
Got2	4	4	4	4
Got3	4	4	4	4
Idh1	4	4	4	4
Idh2	6	6	6	6
Mdh1	6	6	6*	6
Mdh2	3.5	3.5	6	3
Mdh3	16	16	16	16
Mdh4	12	12	12	12
Mdh5	12	12	12	12
Pgm1	9	9	9	9
Pgm2	4	4	4	4
6Pgd1	3.8	3.8	3.8	3.8
6Pgd2	5	5	5	5
Phi1	4	4	4	5

*Allele is probably a 6, but null cannot be ruled out.

The present invention also contemplates a hybrid genetic complement formed by the combination of a haploid genetic complement of the corn plant 87DIA4 with a haploid genetic complement of a second corn plant. Means for combining a haploid genetic complement from the foregoing inbred with another haploid genetic complement can be any method hereinbefore for producing a hybrid plant from 87DIA4. It is also contemplated that a hybrid genetic complement can be prepared using in vitro regeneration of a tissue culture of a hybrid plant of this invention.

A hybrid genetic complement contained in the seed of a hybrid derived from 87DIA4 is a further aspect of this invention. Exemplary hybrid genetic complements are the genetic complements of the hybrid 4033843.

Table 10 shows the identifying numbers of the alleles for the hybrid 4033843, which are exemplary RFLP genetic marker profiles for hybrids derived from the inbred of the present invention. Table 10 concerns 4033843, which has 87DIA4 as one inbred parent.

TABLE 10

RFLP PROFILE FOR 4033843

Probe/Enzyme Combination	Allelic Pair
M0264H	DH
M0306H	AA
M0445E	BC
M1120S	EF
M1234H	AD
M1238H	AE
M1401E	AC
M1406H	AB
M1447H	BB
M1B725E	BB
M2239H	AD
M2297H	AC
M2298E	CC
M2402H	EE
M3212S	AC
M3257S	AB
M3296H	CD
M3432H	AA
M3446S	CF
M3457E	EE
M4386H	BD

TABLE 10-continued

RFLP PROFILE FOR 4033843

Probe/Enzyme Combination	Allelic Pair
M4396E	HH
M4444H	AB
M4UMC19H	AA
M4UMC31S	AD
M5213S	AB
M5408H	AA
M6223E	BC
M6252H	AD
M6280H	EG
M6373E	AE
M7263E	AA
M7391H	AC
M7392S	AC
M7455H	AB
M8110S	AC
M8114E	BB
M8268H	BL
M8585H	AB
M8B2369S	BB
M8UMC48E	CC
M9209E	AC
M9266S	AA
M9B713S	AA
M2UMC34H	DF
M9UMC94H	EE
M3UM121X	CD
M0UMC130	CC

*Probes used to detect RFLPs are from Linkage Genetics, 1515 West 2200 South, Suite C, Salt Lake City, Utah 84119.

The exemplary hybrid genetic complements of hybrid 4033843 may also be assessed by genetic isozyme typing profiles using a standard set of loci as a reference, set, using, e.g., the same, or a different, set of loci to those described above. Table 11 lists the identifying numbers of the alleles at isozyme loci types and presents the exemplary genetic isozyme typing profile for the hybrid 4033843, which is an exemplary hybrid derived from the inbred of the present invention. Table 11 concerns 4033843, which has 87DIA4 as one inbred parent.

TABLE 11

ISOZYME GENOTYPE FOR HYBRID 4033843

LOCUS	ISOZYME ALLELES
Acph1	2
Adh1	4
Cat3	9
Got1	4
Got2	4
Got3	4
Idh1	4
Idh2	6
Mdh1	6
Mdh2	3.5
Mdh3	16
Mdh4	12
Mdh5	12
Pgm1	9
Pgm2	4
6-Pgd1	3.8
6-Pgd2	5
Phi1	4

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of the foregoing illustrative embodiments, it will be apparent to those of skill in the art that variations, changes, modifications and alterations may be applied to the composition, methods, and in the steps or in the sequence of steps of the methods described herein, without departing, from the true concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

Armstrong & Green, "Establishment and Maintenance of Friable Embryogenic Maize Callus and the Involvement of L-Proline," *Planta*, 164:207–214, 1985.

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Jensen, "Chromosome Doubling Techniques in Haploids," Haploids and Higher Plants—*Advances and Potentials, Proceedings of the First International Symposium*, University of Guelph, Jun. 10–14, 1974.

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Rao et al., "Somatic Embryogenesis in Glume Callus Cultures," *Maize Genetics Cooperation Newsletter*, Vol. 60, 1986.

Songstad et al. "Effect of 1-Aminocyclopropate-1-Carboxylic Acid, Silver Nitrate, and Norbornadiene on Plant Regeneration from Maize Callus Cultures," *Plant Cell Reports*, 7:262–265, 1988.

Stuber et al., "Techniques and scoring procedures for starch gel electrophoresis of enzymes of maize *C. Zea mays, L.*," *Tech. Bull.*, N. Carolina Agric. Res. Serv., Vol. 286, 1988.

Wan et al., "Efficient Production of Doubled Haploid Plants Through Colchicine Treatment of Anther-

Derived Maize Callus," *Theoretical and Applied Genetics*, 77:889–892, 1989.

What is claimed is:

1. Inbred corn seed of the corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

2. The inbred corn seed of claim 1, further defined as an essentially homogeneous population of inbred corn seed designated 87DIA4.

3. The inbred corn seed of claim 1, further defined as essentially free from hybrid seed.

4. An inbred corn plant produced by growing the seed of an inbred corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

5. Pollen of the plant of claim 4.

6. An ovule of the plant of claim 4.

7. An essentially homogeneous population of corn plants produced by growing the seed of an inbred corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

8. A corn plant having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

9. The corn plant of claim 8, further comprising a cytoplasmic factor conferring male sterility.

10. A tissue culture of regenerable cells of inbred corn plaint 87DIA4, wherein the tissue regenerates plants having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

11. The tissue culture of claim 10, wherein the regenerable cells are embryos, meristematic cells, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks, stalks, or protoplasts or callus derived therefrom.

12. A corn plant regenerated from the tissue culture of claim 10, having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

13. An inbred corn plant cell of the corn plant of claim 4 having:

(a) an RFLP genetic marker profile in accordance with the profile shown in Table 8; or

(b) a genetic isozyme typing profile in accordance with the profile shown in Table 9.

14. The inbred corn plant cell of claim 13, having an RFLP genetic marker profile in accordance with the profile shown in Table 8.

15. The inbred corn plant cell of claim 13, having a genetic isozyme typing profile in accordance with the profile shown in Table 9.

16. The inbred corn plant cell of claim 13, having an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 8 and 9.

17. The inbred corn plant cell of claim 13, located within a corn plant or seed.

18. The inbred corn plant of claim 4 having:

(a) an RFLP genetic marker profile in accordance with the profile shown in Table 8; or

(b) a genetic isozyme typing profile in accordance with the profile shown in Table 9.

19. The inbred corn plant of claim 18, having an RFLP genetic marker profile in accordance with the profile shown in Table 8.

20. The inbred corn plant of claim 18, having a genetic isozyme typing profile in accordance with the profile shown in Table 9.

21. The inbred corn plant of claim 18, having an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 3 and 9.

22. A process of preparing corn seed, comprising crossing a first parent corn plant with a second parent corn plant, wherein said first or second corn plant is the inbred corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192, wherein seed is allowed to form.

23. The process of claim 22, further defined as a process of preparing hybrid corn seed, comprising crossing a first inbred corn plant with a second, distinct inbred corn plant, wherein said first or second corn plant is the inbred corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

24. The process of claim 23, wherein crossing comprises the steps of:

(a) planting in pollinating proximity seeds of said first and second inbred corn plants;

(b) cultivating the seeds of said first and second inbred corn plants into plants that bear flowers;

(c) emasculating the male flowers of said first or second inbred corn plant to produce an emasculated corn plant;

(d) allowing cross-pollination to occur between said first and second inbred corn plants; and

(e) harvesting seeds produced on said emasculated corn plant.

25. The process of claim 24, further comprising growing said harvested seed to produce a hybrid corn plant.

26. Hybrid corn seed produced by the process of claim 23.

27. A hybrid corn plant produced by the process of claim 25.

28. The hybrid corn plant of claim 27, wherein the plant is a first generation (F_1) hybrid corn plant.

29. The corn plant of claim 8, further comprising a single gene conversion.

30. The corn plant of claim 29, wherein the single gene was stably inserted into a corn genome by transformation.

31. The single gene conversion of the corn plant of claim 29, where the gene is a dominant allele.

32. The single gene conversion of the corn plant of claim 29, where the gene is a recessive allele.

33. The single gene conversion corn plant of claim 29, where the gene confers herbicide resistance.

34. The single gene conversion of the corn plant of claim 29, where the gene confers insect resistance.

35. The single gene conversion of the corn plant of claim 29, where the gene confers resistance to bacterial, fungal, or viral disease.

36. The single gene conversion of the corn plant of claim 29, wherein the gene confers male sterility.

37. The single gene conversion of the corn plant of claim 29, where the gene confers waxy starch.

38. The single gene conversion of the corn plant of claim 29, where the gene confers improved nutritional quality.

39. The single gene conversion of the corn plant of claim 29, where the gene confers enhanced yield stability.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145
DATED : August 10, 1999
INVENTOR(S) : Peter J. Bradbury

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 5, at lines 46-47, please delete "Ear-Fresh Husk The color of the husks 1 to 2 weeks after pollination scored as Color: green, red, or purple" and substitute therefor --Ear-Fresh Husk Color: The color of the husks 1 to 2 weeks after pollination scored as green, red, or purple--.

In col. 5, at lines 56-57, please delete "Ear-Number Per The average number of ears per plant Stalk:" and substitute therefor --Ear-Number Per Stalk: The average number of ears per plant--.

In col. 5, at lines 58-59, please delete "Ear-Shank The average number of internodes on the ear shank. Internodes:" and substitute therefor --Ear-Shank Internodes: The average number of internodes on the ear shank--.

In col. 6, at lines 51-53, please delete "Kernel-Aleurone The color of the aleurone scored as white, pink, tan, brown, Color: bronze, red, purple, pale purple, colorless, or variegated" and substitute therefor --Kernel-Aleurone Color: The color of the aleurone scored as white, pink, tan, brown, bronze, red, purple, pale purple, colorless, or variegated--.

In col. 6, at lines 57-58, please delete "Kernel-Endosperm The color of the endosperm scored as white, pale yellow, or Color: yellow" and substitute therefor --Kernel-Endosperm Color: The color of the endosperm scored as white, pale yellow, or yellow--.

In col. 6, at lines 59-60, please delete "Kernel-Endosperm The type of endosperm scored as normal, waxy, or opaque. Type:" and substitute therefor --Kernel-Endosperm Type: The type of endosperm scored as normal, waxy, or opaque--.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 6, at lines 65-66, please delete "Kernel-Number Per The average number of kernels in a single row. Row:" and substitute therefor --Kernel-Number Per Row: The average number of kernels in a single row--.

In col. 7, at lines 1-3, please delete "Kernel-Pericarp The color of the pericarp scored as colorless, red-white crown, tan, Color: bronze, brown, light red, cherry red, or variegated" and substitute therefor -- Kernel-Pericarp Color: The color of the pericarp scored as colorless, red-white crown, tan, bronze, brown, light red, cherry red, or variegated--.

In col. 7, at lines 4-6, please delete "Kernel-Row The direction of the kernel rows on the ear scored as straight, Direction: slightly curved, spiral, or indistinct (scattered)" and substitute therefor --Kernel-Row Direction: The direction of the kernel rows on the ear scored as straight, slightly curved, spiral, or indistinct (scattered)--.

In col. 7, at lines 30-33, please delete "Leaf-Longitudinal A rating of the number of longitudinal creases on the leaf surface 1 Creases: to 2 weeks after pollination. Creases are scored as absent, few, or many" and substitute therefor --Leaf-Longitudinal Creases: A rating of the number of longitudinal creases on the leaf surface 1 to 2 weeks after pollination. Creases are scored as absent, few, or many--.

In col. 7, at lines 34-36, please delete "Leaf-Marginal A rating of the waviness of the leaf margin 1 to 2 weeks after Waves: pollination. Rated as none, few, or many" and substitute therefor --Leaf-Marginal Waves: A rating of the waviness of the leaf margin 1 to 2 weeks after pollination. Rated as none, few, or many--.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145
DATED : August 10, 1999
INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 7, at lines 40-43, please delete "Leaf-Sheath A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks Anthocyanin: after pollination, scored as absent, basal-weak, basal-strong, weak or strong" and substitute therefor --Leaf-Sheath Anthocyanin: A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks after pollination, scored as absent, basal-weak, basal-strong, weak or strong--.

In col. 7, at lines 44-46, please delete "Leaf-Sheath A rating of the pubescence of the leaf sheath. Ratings are taken 1 Pubescence: to 2 weeks after pollination and scored as light, medium, or heavy" and substitute therefor --Leaf-Sheath Pubescence: A rating of the pubescence of the leaf sheath. Ratings are taken 1 to 2 weeks after pollination and scored as light, medium, or heavy--.

In col. 8, at lines 19-21, please delete "Stalk-Brace Root The color of the brace roots observed 1 to 2 weeks after pollination Color: as green, red, or purple" and substitute therefor --Stalk-Brace Root Color: The color of the brace roots observed 1 to 2 weeks after pollination as green, red, or purple--.

In col. 8, at lines 27-29, please delete "Stalk-Internode The direction of the stalk internode observed after pollination as Direction: straight or zigzag" and substitute therefor --Stalk-Internode Direction: The direction of the stalk internode observed after pollination as straight or zigzag--.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 8, at lines 30-31, please delete "Stalk-Internode The average length of the internode above the primary ear. Length:" and substitute therefor --Stalk Internode Length: The average length of the internode above the primary ear--.

In col. 8, at lines 35-36, please delete "Stalk-Internode With The average number of nodes having brace roots per plant. Brace Roots:" and substitute therefor --Stalk-Internode With Brace Roots: The average number of nodes having brace roots per plant--.

In col. 8, at lines 65-66, please delete "Tassel-Branch The average number of primary tassel branches. Number:" and substitute therefor --Tassel-Branch Number: The average number of primary tassel branches--.

In col. 9, at lines 7-9, please delete "Tassel-Peduncle The average length of the tassel peduncle, measured from the base of the flag leaf to the base Length: of the bottom tassel branch" and substitute therefor --Tassel-Peduncle Length: The average length of the tassel peduncle, measured from the base of the flag leaf to the base of the bottom tassel branch--.

In col. 13, at line 26, delete "3AZA1" and substitute therefor --AQA3--.

In col. 14, at line 52, delete "87DIA114" and substitute therefor --87DIA4--.

In col. 30, at line 4, , delete "DEKALB Plant Genetics" and substitute therefor --DEKALB Genetics Corporation--.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At col. 17, in Table 5 delete all rows under "4. EAR" and ending with "Endosperm Color" and substitute therefor the following rows --

4. EAR				
Silk Color	Pink	Pink	—	Red
Number Per Stalk	1.0	1.0	1.0	1.4
Position (attitude)	Upright	—	—	Upright
Length cm.	14.6	16.0	14.6	15.6
Shape	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Diameter cm.	4.0	3.8	4.0	3.9
Weight gm.	104.9	100.6	103.2	107.6
Shank Length cm.	10.3	14.1	10.1	9.6
Husk Bract	Short	Short	Short	Short
Husk Cover cm.	6.4	2.5	4.4	3.7
Husk Color Fresh	Green	Green	Green	Green
Husk Color Dry	Buff	Buff	Buff	Buff
Cob Diameter cm.	2.3	2.4	2.3	2.1
Cob Color	Red	—	Red	Red
Shelling Percent	87.7	80.6	89.0	83.3

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

5. KERNEL

Row Number	14.6	14.8	16.3	15.3
Number Per Row	32.1	27.1	29.3	29.7
Row Direction	Curved	Curved	Curved	Curved
Type	Dent	—	Dent	—
Cap Color	Yellow	Yellow	Yellow	Yellow
Side Color	Deep Yellow	—	Orange	
Length (depth) mm.	11.1	9.4	10.9	10.3
Width mm.	7.8	8.0	7.4	7.8
Thickness	3.9	5.2	4.4	4.2
Weight of 1000K gm.	269.0	252.4	233.0	247.8
Endosperm Type	Normal	Normal	Normal	Normal
Endosperm Color	Yellow	Yellow	Yellow	Yellow--

Signed and Sealed this

Twenty-seventh Day of February, 2001

Attest:

Nicholas P. Godici

NICHOLAS P. GODICI

Attesting Officer *Acting Director of the United States Patent and Trademark Office*

EXHIBIT C

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte JAMES R. LARKINS, DAVID D. SONGSTAD, WILLIAM L. PETERSEN, HONGYI ZHANG, MICHAEL T. MANN, MICHAEL SPENCER, and NANCY G. WILLETTS

MAILED
APR 07 2005
U.S. PATENT AND TRADEMARK OFFICE
BOARD OF PATENT APPEALS
AND INTERFERENCES

Appeal No. 2004-1503[1]
Application No. 09/606,808

~~MAILED~~
~~MAR 31 2005~~
~~U.S. PATENT AND TRADEMARK OFFICE~~
~~BOARD OF PATENT APPEALS~~
~~AND INTERFERENCES~~

ON BRIEF

RECEIVED
Date(s) Docketed: Bd. Decision Rec'd; Rejections Reversed; 7/11/05 Status Check for Not. of Allowance
APR 11 2005
Client: DEKA:264
Attorney(s): DLP/REH
Initials: 4/14/05

Before SCHEINER, ADAMS and GREEN, Administrative Patent Judges.

ADAMS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on the appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 2, 3, 7-17 and 22-39. The examiner has indicated that claims 1, 4-6 and 18-21 are allowable. Answer, page 2.

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[1] This appeal is substantially similar to Appeal No. 2004-1506, Application No. 09/788,334; Appeal No. 2004-1968, Application No. 10/00,0311; Appeal No. 2004-2317, Application No. 09/771,938; Appeal No. 2004-2343, Application No. 09/772,520; and Appeal No. 2005-0396, Application No. 10/077,589, which all share the same assignee, Monsanto Company, the parent of wholly-owned subsidiary DeKalb Genetics Corporation. Accordingly we have considered these appeals together.

Claims 2, 8-10, 14, 25, 26, 29 and 30 are illustrative of the subject matter on appeal and are reproduced below. In addition, for convenience, we have reproduced allowable claims 1 and 4 below:

1. Inbred corn seed of the corn plant LIZL5, a sample of said seed having been deposited under ATCC Accession No. PTA-2192.

2. The inbred corn seed of claim 1, further defined as an essentially homogeneous population of inbred corn seed.

4. An inbred corn plant produced by growing the seed of the inbred corn plant LIZL5, a sample of said seed having been deposited under ATCC Accession No. PTA-2129.

8. A corn plant capable of expressing all the physiological and morphological characteristics of the inbred corn plant LIZL5, a sample of the seed of said inbred corn plant LIZL5 having been deposited under ATCC Accession No. PTA-2192.

9. The corn plant of claim 8, further comprising a cytoplasmic or nuclear gene conferring male sterility.

10. A tissue culture of regenerable cells of inbred corn plant LIZL5, wherein the tissue regenerates plants capable of expressing all the physiological and morphological characteristics of the inbred corn plant LIZL5, a sample of the seed of said corn plant LIZL5 having been deposited under ATCC Accession No. PTA-2192.

14. An inbred corn plant cell of the corn plant of claim 8, said cell comprising:
    (a) an RFLP genetic marker profile[2] in accordance with the profile shown in Table 6; or
    (b) a genetic isozyme typing profile in accordance with the profile shown in Table 7.

25. The corn plant of claim 4, further comprising a single locus conversion.

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[2] We note that claims 14 and 17 appear to include a typographical error in reference to RFLP genetic marker profiles. As the examiner points out (Answer, page 12), "Table 6 in the specification actually shows SSR profiles, not RFLP genetic marker profiles as indicated in claims 14 and 17." Therefore, prior to any further action on the merits, we encourage the examiner and appellants to clarify this issue on the record.

26. The corn plant of claim 25, wherein the single locus was stably inserted into a corn genome by transformation.

29. A method of preparing a transgenic maize cell comprising:
    a) Providing cells of inbred corn plant LIZL5, a sample of the seed of the inbred LIZL5 having been deposited under ATCC Accession No. PTA-2192;
    b) Contacting said cells with a pre-selected DNA; and
    c) Identifying at least a first transgenic cell of inbred corn plant LIZL5 which has been transformed with said pre-selected DNA.

30. The method of claim 29, further comprising the step of:
    d) Regenerating a fertile transgenic plant from said transgenic cell.

The references relied upon by the examiner are:

Hunsperger et al. (Hunsperger)	5,523,520	Jun. 4, 1996

Eshed et al. (Eshed), "Less-Than-Additive Epistatic Interactions of Quantitative Trait Loci in Tomato," Genetics, Vol. 143, pp. 1807-17 (1996)

Kraft et al. (Kraft), "Linkage Disequilibrium and Fingerprinting in Sugar Beet," Theoretical and Applied Genetics, Vol. 101, pp. 323-36 (2000)

GROUNDS OF REJECTION

Claim 2 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "an essentially homogeneous population of inbred corn seed."

Claim 3 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "essentially free from hybrid seed."

Claim 7 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "[a]n essentially homogeneous population of corn plants produced by growing the seed of the inbred corn plant LIZL5."

Claims 8, 10-13[3] and 37 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "capable of expressing," as it is used in claims 8 and 10-13; and the term "preparable," as it is used in claim 37.

Claims 9 and 25 stand rejected under 35 U.S.C. § 112, second paragraph as failing to limit the scope of the independent claim from which they depend.

Claims 14 and 17 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "in accordance with."

Claim 25 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of "comprising a single locus conversion."

Claim 26 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the article 'a' in the recitation 'wherein the single locus was stably inserted into a corn genome.'"

Claim 28 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrases "yield enhancement," "improved nutritional quality," and "enhanced yield stability."

Claims 14-17 and 22-39 stand rejected under the written description provision of 35 U.S.C. § 112, first paragraph.

Claims 25-39 stand rejected under the enablement provision of 35 U.S.C. § 112, first paragraph.

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[3] We note the examiner's assertions (Answer, page 11), "claim 11 remains rejected because it depends from claim 10," and "claim 12 remains rejected because it depends from claim 11." Accordingly, we have included claims 11 and 12 in the statement of this rejection.

We reverse.

BACKGROUND

The present "invention relates to inbred corn seed and plants designated LIZL5, and derivatives, tissue cultures thereof, methods of transformation of plants or parts thereof of the plant designated LIZL5 and transformants derived thereof." Specification, page 1. According to appellants (specification, page 25), "[a] description of the physiological and morphological characteristics of corn plant LIZL5 is presented in Table 3" of the specification, pages 25-27. On this record the examiner has indicated that claims drawn to plants, plant parts, and seed of the corn variety designated LIZL5 are allowable. See e.g., claims 1, and 4-6, and Answer, page 2, wherein the examiner states "[c]laims 1, [and] 4-6 ... are allowed."

A second aspect of the present invention comprises hybrid plants and processes "for producing [first generation (F_1) hybrid[4]] corn seeds or plants, which ... generally comprise crossing a first parent corn plant with a second

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[4] We recognize the examiner's statement (Answer, page 3), "[c]laim 24 was objected to in the Office [A]ction mailed 16 July 2003, as being in improper dependent form for failing to further limit the subject matter of previous claim. Appellants did not address this objection." An objection to a claim, however, is the subject matter of a petition, and is not properly before us on appeal. Nevertheless, we make the following observation regarding claim 24, and encourage the examiner and appellants to work together to remedy this issue, prior to any further action on the merits.

According to appellants' specification (page 19), a F_1 hybrid is "[t]he first generation progeny of the cross of two plants." Therefore, as we understand the prosecution history as well as the language of the claims, claims 22 and 23 to refer to F_1 hybrids. In this regard, we note that similar claims, directed to a different corn variety, were presented for our review in Appeal Nos. 2004-1506 and 2004-2317. During the oral hearing in Appeal Nos. 2004-1506 and 2004-2317, appellants' representative confirmed that all claims drawn to hybrid plants or hybrid seeds (see e.g., claims 24 and 25 of Appeal Nos. 2004-1506 and 2004-2317) refer to F_1 hybrids. Accordingly, it appears that claim 24 fails to further limit claim 23 from which it depends.

parent corn plant, wherein at least one of the first or second parent corn plants is the inbred corn plant designated LIZL5." Specification, pages 7-8. On this record the examiner has indicated that claims drawn to a process of producing corn seed wherein the process comprises crossing a first parent corn plant with a second parent corn plant are allowable. See e.g., claims 18-21 and Answer, page 2, wherein the examiner states claims "18-21 are allowed."

A third aspect of the present invention comprises single locus converted plants of the corn variety LIZL5. Specification, page 6. As appellants explain (specification, page 21, emphasis added), single locus converted (conversion) plants are those plants

> which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. A single locus may comprise one gene, or in the case of transgenic plants, one or more transgenes integrated into the host genome at a single site (locus).

As appellants explain (specification, page 29):

> Many single locus traits have been identified that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques. Single locus traits may or may not be transgenic; examples of these traits include, but are not limited to, male sterility, waxy starch, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, male fertility, enhanced nutritional quality, industrial usage, yield stability, and yield enhancement. These genes are generally inherited through the nucleus, but may be inherited through the cytoplasm. Some known exceptions to this are genes for male sterility, some of which are inherited cytoplasmically, but still act as single locus traits.

A final aspect of the present invention is directed to a method of preparing a transgenic maize cell comprising the use of cells of inbred corn plant LIZL5. See e.g., claim 29. According to appellants' specification (page 10), the "invention provides a method of preparing a transgenic maize cell comprising: a) providing cells of inbred corn plant LIZL5, b) contacting the cells with a pre-selected DNA; and c) identifying at least a first transgenic cell of inbred corn plant LIZL5 which has been transformed with the pre-selected DNA."

Against this backdrop, we now consider the rejections of record.

DISCUSSION

Definiteness:

Claims 2, 3, 7-17, 25-28, and 37-39 stand rejected under 35 U.S.C. § 112, second paragraph. For the following reasons we reverse.

Claim 2

Claim 2 depends from independent claim 1, and stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "an essentially homogeneous population of inbred corn seed...." Answer, page 4. According to the examiner (id.), "[g]iving claim 2 its plain meaning, the inbred corn seed of claim 1 must, by definition, be a homogeneous population." Thus, the examiner finds (id.), the "'essentially homogeneous' language [in claim 2] ... appear[s] to be superfluous."

However, as disclosed in appellants' specification (page 5),

> [e]ssentially homogeneous populations of inbred seed are those that consist essentially of the particular inbred seed, and are generally free from substantial numbers of other seed, so that the

> inbred seed forms between about 90% and about 100% of the total seed, and preferably, between about 95% and about 100% of the total seed.

Accordingly, we disagree with the examiner's assertion (Answer, page 6) that claim 2 is unclear simply because it may contain seed other than the seed of the corn variety LIZL5. We remind the examiner that claim language must be analyzed "not in a vacuum, but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary skill in the pertinent art." In re Moore, 439 F.2d 1232, 1235, 169 USPQ 236, 238 (CCPA 1971). Accordingly, it is our opinion that a person of ordinary skill in the art would recognize that an essentially homogeneous population of seed of the corn variety LIZL5 is a population of seed that is generally free from substantial numbers of other seed, e.g., wherein corn variety LIZL5 seed forms between about 90% and about 100% of the total seed in the population.[5]

Accordingly, we reverse the rejection of claim 2 under 35 U.S.C. § 112, second paragraph.

Claim 3

According to the examiner (Answer, page 5), claim 3 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "essentially free from hybrid seed," "for reasons similar to the rejection of

[5] Cf. the examiner's statement (Answer, page 6), "if claim 2 were amended to read '[a]n essentially homogeneous population of corn seeds consisting essentially of seed the inbred corn seed of claim 1', the claim would have a definite meaning.

claim 2. Thus, the examiner recommends (id.), claim 3 be amended to read, "[a] population of corn seeds consisting essentially of the inbred corn seed of claim 1, and essentially free from hybrid seeds." Therefore, for the reasons, set forth in our discussion of the rejection of claim 2 under 35 U.S.C. § 112, second paragraph above, we agree with appellants (Brief, page 5), claim 3 "further defines ... claim [1] from which it depends by requiring that the seed be free of hybrid seed."

Accordingly, we reverse the rejection of claim 3 under 35 U.S.C. § 112, second paragraph.

Claim 7

Claim 7 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "[a]n essentially homogeneous population of corn plants produced by growing the seed of the inbred corn plant LIZL5...." Answer, page 6. According to the examiner (id.), "LIZL5 seed can only produce LIZL5 plants. ... [Therefore,] [t]he population can ... only consist of LIZL5 plants." Accordingly, the examiner finds it unclear "why the population is referred to as 'essentially homogeneous,' since such populations can comprise more than one variety of plant." Id.

As appellants disclose (specification, page 6), "[t]he population of inbred corn seed of the invention can further be particularly defined as being essentially free from hybrid seed. The inbred seed population may be separately grown to provide an essentially homogeneous population of inbred corn plants designated LIZL5." As we understand the claim, growing the seed of claim 3, for example,

would produce an essentially homogeneous population of corn plants of the corn variety LIZL5.[6]

In addition, we direct the examiner's attention to Appeal No. 2005-0396, wherein a claim similar to claim 7 was presented for our review. In Appeal No. 2005-0396, the examiner of record indicated that claim 14, directed to "[a]n essentially homogeneous population of corn plants produced by growing the seed of the corn variety I180580...." was allowable. Accordingly, we find that the examiner has treated claim 7 in a manner that is inconsistent with the prosecution of claim 14 in 2005-0396. As we understand it, the only difference between claim 14 as it appears in Appeal No. 2005-0396 and claim 7 before us in the instant appeal is the variety of corn seed from which the plant is produced.

Accordingly we reverse the rejection of claim 7 under 35 U.S.C. § 112, second paragraph.

Claims 8, 10-13 and 37

Claims 8, 10-13 and 37 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "capable of expressing," as it is used in claims 8 and 10-13; and the term "preparable" as it is used in claim 37. According to the examiner (Answer, page 8), the recitation of the phrase "capable of" in claims 8 and 10-13 "does not make clear if the plant actually

---

[6] Cf. The examiner's statement (Answer, page 7), "[a]mending claim 7 to read, '[a]n essentially homogeneous population of corn plants produced by growing a population of corn seed consisting essentially of the seed of corn plant LIZL5...' would obviate this rejection."

expresses the traits, or when or under what conditions the traits are expressed."

In this regard, the examiner finds (Answer, page 9),

> while the plant has the capacity to express the characteristics, for some reason it may not. Certain characteristics of a plant are expressed only at certain times of its life cycle, and are incapable of being expressed at other times. The colors of flower parts such as silks, or fruit parts such as husks, are examples. The promoters of many genes conferring traits require a transcription factor to become active. Is a plant that has such a gene, but not the transcription factor, considered "capable of expressing" that gene, and the trait associated with that gene, and is such a plant encompassed by the claims?

To address the examiner's concerns, we find it sufficient to state that if a plant has the capacity to express the claimed characteristics it meets the requirement of the claim regarding "capable of," notwithstanding that due to a particular phase of the life cycle the plant is not currently expressing a particular characteristic. Alternatively, if a plant is incapable of expressing the claimed characteristics at any phase of the life cycle, because it lacks, for example, the "transcription factor" required for expression – such a plant would not meet the requirement of the claim regarding "capable of."

Here, we find the examiner's extremely technical criticism to be a departure from the legally correct standard of considering the claimed invention from the perspective of one possessing ordinary skill in the art.[7] In our opinion, a person of ordinary skill in the art would understand what is claimed. Amgen Inc. v. Chugai Pharmaceutical Co., Ltd., 927 F.2d 1200, 1217, 18 USPQ2d 1016, 1030 (Fed. Cir. 1991).

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[7] Cf. Digital Equipment Corp. v. Diamond, 653 F.2d 701, 724, 210 USPQ 521, 546 (CA 1981).

Similarly, the examiner finds (Answer, page 16), the recitation of the term "preparable" in claim 37 "leaves open the possibility that the claimed fertile transgenic corn plant can be prepared by any other means." In our opinion, like "capable of" above, the claim requires that the plant be able to be produced by the process of claim 30. That a person of ordinary skill in the art may conceive of other ways to prepare the plant is of no concern. Alternatively, if a plant cannot be prepared according to the process of claim 30 it would be outside the scope of claim 37.

Accordingly we reverse the rejection of claims 8, 10-13 and 37 under 35 U.S.C. § 112, second paragraph.

<u>Claims 9 and 25</u>

Claim 9 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "a cytoplasmic or nuclear gene conferring male sterility. As we understand the examiner's argument (Answer, bridging paragraph, pages 9-10), since the plant set forth in claim 9 is male sterile it "cannot incorporate all the limitations of claim 8…" from which claim 9 depends. Similarly, the examiner finds it unclear whether the plant set forth in claim 25 has all the traits expressed by the plant of … [claim] 5," from which claim 25 depends. Answer, page 13. In response, appellants assert (Brief, pages 7 and 9), claims 9 and 25 simply add a further limitation to the claims from which they depend. We agree.

For example, claim 9 reads on a corn plant capable of expressing all the physiological and morphological characteristics of the corn variety LIZL5, further

comprising a nuclear or cytoplasmic gene conferring male sterility. In our opinion, the claim reasonably apprises those of skill in the art of its scope. Amgen, In our opinion, the same is true of claim 25. As set forth in Shatterproof Glass Corp. v. Libbey-Owens Ford Co., 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir. 1985), "[i]f the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more."

Accordingly we reverse the rejection of claims 9 and 25 under 35 U.S.C. § 112, second paragraph.

Claims 14 and 17

Claims 14 and 17 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "in accordance with." According to the examiner (Answer, page 12), it is unclear if a plant "that generally follows the trend of the profile of Table 6, but which differs at one or a few loci, [would] be considered in 'conformity' or 'in accordance' with the profile of Table 6."

On this record, we understand the phrase "in accordance with" as it is used in claims 14 and 17 to mean "the same"[8]. Furthermore, as discussed supra, n. 2, claims 14 and 17 appear to include a typographical error in reference

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[8] Cf. Appeal Nos. 2004-1506 and 2004-2317, which use similar language for claims directed to different corn varieties. In this regard, we note that during the February 10, 2005 oral hearing in Appeal Nos. 2004-1506 and 2004-2317, appellants' representative confirmed that the phrase "in accordance with" was intended to mean "the same".

to RFLP genetic marker profiles of Table 6, whereas, Table 6 of appellants' specification illustrates SSR profiles, not RFLP genetic marker profiles. Stated differently, we understand the claims to read:

> 15. An inbred corn plant cell of the corn plant of claim 8, said cell comprising:
> (c) the same SSR profile as shown in Table 6; or
> (d) the same genetic isozyme typing profile as shown in Table 7.
>
> 17. The inbred corn plant of claim 8, comprising:
> (a) the same SSR profile as shown in Table 6; or
> (b) the same genetic isozyme typing profile as shown in Table 7.

Accordingly we reverse the rejection of claims 14 and 17 under 35 U.S.C. § 112, second paragraph.

Claim 26

Claim 26 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the article 'a' in the recitation 'wherein the single locus was stably inserted into a corn genome.'" According to the examiner (Answer, page 14), "[t]he recitation does not make clear if the genome is that of LIZL5 or that of a different corn plant."

According to appellants' specification (page 21, emphasis removed), a "Single Locus Converted (Conversion) Plant" refers to

> [p]lants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. A single locus may comprise one gene, or in the case of transgenic plants, one or more transgenes integrated into the host genome at a single site (locus).

Accordingly, we agree with appellants (Brief, bridging paragraph, pages 10-11), the single locus referred to in claim 26 may or may not have been directly inserted into the genome of the claimed plant. As we understand the claim, and arguments of record, claim 26 presents two possibilities: (1) the single locus is directly inserted into the claimed plant and nothing further need be done; or (2) the single locus is directly inserted into a different plant, which is then used to transfer the single locus to the claimed plant through use of the plant breeding technique known as backcrossing.

In our opinion, the claim reasonably apprises those of skill in the art of its scope. Amgen. Accordingly, we reverse the rejection of claim 26 under 35 U.S.C. § 112, second paragraph.

Claim 28

Claim 28 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrases "yield enhancement," "improved nutritional quality," and "enhanced yield stability." According to the examiner the terms "yield enhancement," "improved nutritional quality," and "enhanced yield stability" are relative and have no definite meaning. Answer, page 14.

On this record, appellants assert (Brief, page 13), it is "understood the enhancement of yield or yield stability and improved nutritional quality is relative to a plant lacking the single locus. The metes and bounds of the claim are thus fully understood by one of skill in the art and the use of the terms is not indefinite." On reflection, we agree with appellants. The fact that some claim language is not mathematically precise does not per se render the claim

indefinite. Seattle Box. Co. v. Industrial Crating & Packing, Inc., 731 F.2d 818, 826, 221 USPQ 568, 573-574 (Fed. Cir. 1984). As set forth in Shatterproof Glass, "[i]f the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more." In our opinion, a person of ordinary skill in the art would have understood the enhancement of yield or yield stability and improved nutritional quality is relative to a plant lacking the single locus.

Accordingly we reverse the rejection of claim 28 under 35 U.S.C. § 112, second paragraph.

Written Description:

Claims 14-17 and 22-39 stand rejected under 35 U.S.C. § 112, first paragraph, as the specification fails to adequately describe the claimed invention. For the following reasons, we reverse.

Claims 22-24

Claims 22-24 both ultimately depend from claim 18. On this record, the examiner has indicated that claims 18-21 are allowable. Answer, page 2. The examiner finds (Answer, page 18), claims 22-24 are drawn to a hybrid plant or seed "produced by crossing inbred corn plant LIZL5 with any second, distinct inbred corn plant."

As we understand it, based on this construction of claims 22-24, the examiner is of the opinion that since the hybrids inherit only ½ of their diploid[9] set of chromosomes from the plant of corn variety LIZL5, a person of skill in the art would not have viewed the teachings of the specification as sufficient to demonstrate that appellants were in possession of the genus of hybrid seeds and plants encompassed by claims 22-24. See Answer, pages 23-24. According to the examiner (Answer, page 24), "[t]he fact that any hybrid plant will inherit half of its alleles from LIZL5 then does not provide sufficient description of the morphological and physiological characteristics expressed by the claimed hybrid plants."

There is no doubt that the expressed gene products of a hybrid plant, e.g., the morphological and physiological traits, of LIZL5 and a non-LIZL5 corn plant will depend on the combination of the genetic material inherited from both parents. See Answer, page 24. Nevertheless, we disagree with the examiner's conclusion (id.) that "[t]he fact that any hybrid plant will inherit half of its alleles from LIZL5 then does not provide sufficient description of the morphological and physiological characteristics expressed by the claimed hybrid plants."

On these facts, we find it necessary to take a step back and consider what is claimed. The claims are drawn to a F_1 hybrid seed (claim 22) or plant (claim 23) resulting from a cross between a plant of corn variety LIZL5 and a non-LIZL5 corn variety. The claims do not require the hybrid to express any particular

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[9] According to appellants' specification (page 19), diploid means "a cell or organism having two sets of chromosomes."

morphological or physiological characteristic. Nor do the claims require that a particular non-LIZL5 corn variety be used.[10] All that is required by the claims is that the hybrid has one parent that is a plant of corn variety LIZL5. Since the examiner has indicated that the seed and the plant of the corn variety LIZL5 are allowable (see claims 1 and 4), there can be no doubt that the specification provides an adequate written description of this corn variety. In addition, the examiner appears to recognize (see e.g., Answer, page 17) that appellants' specification describes an exemplary hybrid wherein one parent was a plant of the corn variety LIZL5. Accordingly, it is unclear to this merits panel what additional description is necessary.

As set forth in Reiffin v. Microsoft Corp., 214 F.3d 1342, 1345, 54 USPQ2d 1915, 1917 (Fed. Cir. 2000), the purpose of the written description requirement is to "ensure that the scope of the right to exclude, as set forth in the claims does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification." Here the hybrid seed or plant has one parent that is a plant of the corn variety LIZL5. To that end, to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). For the foregoing

[10] According to appellants (Brief, page 14), "hundreds or even thousands of different inbred corn lines were well known to those of skill in the art prior to the filing [date] of the instant application, each of which could be crossed to make a hybrid plant with in the scope of the claims."

reasons it is our opinion that appellants have provided an adequate written description of the subject matter set forth in claims 24-26.[11]

We recognize the examiner's argument relating to SSR and isozyme markers (Answer, pages 26-27), as well as the examiner's arguments concerning a correlation between the hybrid's genome structure and the function of the hybrid plant (Answer, page 25). However, for the foregoing reasons, we are not persuaded by these arguments.[12]

Claims 14-17

According to the examiner (Answer, page 19), while the specification provides the locus names and allele numbers of the SSR markers, the specification does not provide the actual nucleotide sequences that make up the markers. According to the examiner (id.), "names of loci alone do not describe the structures of the markers themselves. Without a description of the sequences of the markers, one cannot confirm their presence." However, as the examiner recognizes (id.), "[t]he specification indicates on page 57, lines 17-18, that the SSR analyses were conducted at Celera AgGen, and on page 60, line 3, that primers used in the analyses are also from Celera AgGen." In this regard, appellants point out (Brief, page 15), "the service that was used to detect SSR markers is commercially available to the public." In other words, a person of

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[11] Again, we note as set forth in n. 4, claim 24 does not appear to further limit the scope of claim 23 from which it depends.

[12] For the same reasons we are not persuaded by the examiner's assertion (Answer, page 22) that appellants' specification fails to provide an adequate written description of claims 38 and 39.

ordinary skill in the art could use the commercially available service provided by Celera AgGen, Inc. to determine whether a corn plant produced by growing a seed of the corn variety LIZL5 has an SSR profile which is the same as that shown in Table 6. Therefore, it is unclear to this panel why the examiner believes that such a disclosure fails to provide adequate written descriptive support for the claimed invention.[13] Accordingly, we are not persuaded by the examiner's argument.

Regarding the isozyme typing profile, the examiner notes (Answer, page 19), "16 of the 18 isozyme markers of LIZL5 in Table 7 are also found in at least two other corn varieties, those of the other plants of Table 7." Based on this observation, the examiner concludes (id., emphasis added), "the markers in Table 6 are not adequate to distinguish the claimed hybrids from other corn plants, as other corn plants contain almost all of the same markers.[14]" We find the examiner's logic somewhat inconsistent, the examiner recognizes that isozyme typing profiles of "other corn plants" are different, yet concludes that the different isozyme profiles are inadequate to distinguish the claimed hybrids from other corn plants. Accordingly, we are not persuaded by the examiner's argument.

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[13] We are not persuaded by the examiner's assertion (Answer, page 30) "that the [commercially available] service used to detect SSR markers is currently available is not a guarantee that it will remain so for the life of a patent issuing from the application." Cf. In re Metcalfe, 410 F.2d 1378, 1382, 161 USPQ 789, 792-3 (CCPA 1969).

[14] Stated differently, the examiner recognizes that the isozyme typing profiles of the corn plants are different.

In addition, we direct the examiner's attention to claims 6 and 11 of Appeal No. 2005-0396. As we understand it, notwithstanding differences in the SSR and isozyme profiles, the disclosure in the specification as well as the language of the claims is substantially similar to that of the instant application. Nevertheless, the examiner in Appeal No. 2005-0396 apparently found that appellants' specification provided an adequate written description of the claimed invention as no rejection of claims 6 and 11 was made under the written description provision of 35 U.S.C. § 112, first paragraph in Appeal No. 2005-0396. Accordingly, we find that the examiner has treated claims 14-17 in a manner that is inconsistent with the prosecution of similar claims in related application 10/077,589, which is the subject matter of Appeal No. 2005-0396.

For the foregoing reasons, we are not persuaded by the examiner's arguments.

Claims 25-28

According to the examiner (Answer, page 20), "[c]laims 25-28 are drawn towards LIZL5 plants further comprising a single locus conversion, or wherein the single locus was stably inserted into a corn genome by transformation." The examiner finds, however, that "the specification does not describe identified or isolated single loci for all corn plant traits." Id. More specifically, the examiner finds (id.), claims 25-27 "broadly encompass single loci that have not been discovered or isolated." To the extent that the examiner is asserting that appellants have not provided an enabling disclosure of single loci that have not been identified, we note that to satisfy the written description requirement, the

inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath.

Nevertheless, it may be that the examiner's concern (Answer, page 34), is that "single genes that alone govern 'yield enhancement' or 'enhanced yield stability' have not been discovered." The examiner, however, provides no evidence to support the assertion that a person of ordinary skill in the art would not recognize that single loci for yield enhancement or yield stability are known in the art. In this regard, we note that appellants disclose (specification, page 29), "[m]any single locus traits have been identified ... examples of these traits include, but are not limited to, ... enhanced nutritional quality, industrial usage, yield stability, and yield enhancement." It appears that the examiner has overlooked appellants' assertion that single locus traits for yield stability and yield enhancement are well known in the art. To this end, we direct the examiner's attention to, for example, United States Patent No. 5,936,145 ('145)[15], issued August 10, 1999, which is prior to the filing date of the instant application. For clarity, we reproduce claims 8, 29 and 39 of the '145 patent below:

8. A corn plant having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

29. The corn plant of claim 8, further comprising a single gene conversion.

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[15] We note that the assignee of the '145 patent is DeKalb Genetics Corporation. The assignee of the present application is Monsanto Company, the parent of wholly-owned subsidiary DeKalb Genetics Corporation.

39. The single gene conversion of the corn plant of claim 29, where the gene confers enhanced yield stability.

As we understand it, claim 39 of the '145 patent, is drawn to a corn plant which comprises a single gene conversion, wherein the gene confers enhanced yield stability. Thus, contrary to the examiner's assertion it appears, for example, that a single gene that confers enhanced yield stability was known in the art prior to the filing date of the instant application. We remind the examiner "a patent need not teach, and preferably omits, what is well known in the art." Hybritech Incorporated v. Monoclonal Antibodies, Inc. 802 F.2d 1367, 1385, 231 USPQ 81, 94 (Fed. Cir. 1986).

We remind the examiner that the inquiry into whether the description requirement is met must be determined on a case-by-case basis and is a question of fact. In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). A description as filed is presumed to be adequate; unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. See e.g., In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. Accordingly, it is the examiner who has the initial burden of establishing by a preponderance of evidence that a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. Wertheim, 541 F.2d at 263, 191 USPQ at 97. On this record, the examiner

provides no evidence to support the assertion that single loci that govern, for example, yield enhancement or enhanced yield stability are not described.

For the foregoing reasons, we are not persuaded by the examiner's arguments.[16]

Summary

For the foregoing reasons, we reverse the rejection of claims 14-17 and 22-39 under the written description provision of 35 U.S.C. § 112, first paragraph.

Enablement:

Claims 25-39 stand rejected under the enablement provision of 35 U.S.C. § 112, first paragraph. The examiner finds (Answer, page 36), claims 27-30

> are broadly drawn to inbred corn plant LIZL5 further comprising a single locus conversion; or to any method of preparing transgenic LIZL5 cells comprising contacting cells of inbred corn plant LIZL5 with any pre-selected DNA, having any function; or wherein said method further comprises regenerating a fertile transgenic plant; or a fertile transgenic plant produced by said method; or seed of said transgenic plant; or a plant grown from said seed.

According to the examiner (Answer, page 37), "[a] review of claim 25 indicates that it encompasses corn plant LIZL5, and therefore all of its morphological and physiological traits, and further comprising any single locus." While the examiner recognizes (id.), "[t]he practice of crossing two plant varieties, each expressing two different desired traits ... is well-established," the examiner finds (id.), "the specification does not teach any LIZL5 plants

[16] For the same reasons we are not persuaded by the examiner's assertion (Answer, page 22) that appellants' specification fails to provide an adequate written description of claims 29-39.

comprising a single locus conversion produced by backcrossing, wherein the resultant plant retains all of its morphological and physiological traits in addition to exhibiting the single trait conferred by the introduced single locus." With reference to Hunsperger, Kraft, and Eshed, the examiner asserts (Answer, page 42), "[t]he rejection raises the issue of how linkage drag hampers the insertion of single genes alone into a plant by backcrossing, while recovering all of the original plant's genome."

Notwithstanding the examiner's assertions to the contrary, claims 25-39 do not require that the single locus conversion plant retain all of the morphological and physiological traits of the parent plant in addition to exhibiting the single trait conferred by the introduction of the single loci. Nor do claims 25-39 require that the resultant plant retain all of the original plant's genome in addition to the single locus transferred into the inbred via the backcrossing technique. As appellants explain (specification, bridging paragraph, pages 27-28, emphasis added),

> [t]he term single locus converted plant as used herein refers to those corn plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single locus transferred into the inbred via the backcrossing technique.

See also appellants' definition of single locus converted (conversion) plant at page 23 of the specification. We find nothing in the appellants' specification to indicate that the single locus converted plant retains all of the morphological and physiological traits, or all of the genome, of the parent plant in addition to the

single locus transferred via the backcrossing technique. Accordingly, we disagree with the examiner's construction of claims 25-39 as "directed to exactly plant LIZL5, further comprising the single locus.," which appears to disregard appellants' definition of a single locus converted plant. See Answer, page 43.

The examiner appreciates (Answer, page 37) that appellants' specification provides an example of a converted plant. While the examiner finds (id.), that this converted plant was not a LIZL5 converted plant, the examiner offers no evidence on this record that similar methodology used to produce the exemplified converted plant would not also be effective in producing a LIZL5 converted plant. Nor did the examiner provide evidence that the converted plant exemplified in appellants' specification did not retain essentially all of the desired morphological and physiological characteristics of the inbred in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique.

Further, we recognize appellants' argument (Brief, page 24) that the examiner failed to establish a nexus between Hunsperger's discussion of petunias; Kraft's discussion of sugar beets; and Eshed's discussion of tomatoes, and the subject matter of the instant application - corn. Absent evidence to the contrary, we agree with appellants (id.), "[t]he [examiner's] indication[17] that the references concerning petunias, sugar beets and tomatoes apply to corn is made without any support." That the examiner has failed to identify (Answer,

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[17] See Answer page 42, wherein the examiner asserts "[l]inkage drag appears to be a phenomenon that occurs in all plant types."

page 42) an example "in the prior art of plants in which linkage drag does not occur," does not mean that linkage drag is expected to occur in corn breeding, which according to appellants (Reply Brief, page 10) "is extremely advanced and well known in the art...." In this regard, we agree with appellants (Brief, bridging paragraph, pages 24-25; Accord Reply Brief, pages 7-8), the examiner has improperly placed the burden on appellants to demonstrate that the examiner's unsupported assertion is not true. We remind the examiner, as set forth in In re Wright, 999 F.2d 1557, 1561-62, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993):

> When rejecting a claim under the enablement requirement of section 112, the PTO bears an initial burden of setting forth a reasonable explanation as to why it believes that the scope of protection provided by that claim is not adequately enabled by the description of the invention provided in the specification of the application; this includes, of course, providing sufficient reasons for doubting any assertions in the specification as to the scope of enablement.

For the foregoing reasons, we reverse the rejection of claims 25-39 under the enablement provision of 35 U.S.C. § 112, first paragraph.

SUMMARY

We reverse the rejection of claims 2, 3, 7-17, 25-28, and 37-39 under 35 U.S.C. § 112, second paragraph.

We reverse the rejection of claims 14-17 and 22-39 under the written description provision of 35 U.S.C. § 112, first paragraph.

We reverse the rejection of claims 25-39 under the enablement provision of 35 U.S.C. § 112, first paragraph.

We do not reach the merits of the objection to claim 24, which was not presented for our review.

REVERSED

Toni R. Scheiner Administrative Patent Judge	
Donald E. Adams Administrative Patent Judge	BOARD OF PATENT APPEALS AND INTERFERENCES
Lora M. Green Administrative Patent Judge	

Robert E. Hanson
FULBRIGHT & JAWORSKI L.L.P.
A REGISTERED LIMITED LIABILITY PARTNERSHIP
600 CONGRESS AVENUE, SUITE 2400
AUSTIN, TX 78701

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*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-5,936,145	08-1999	Bradbury	
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

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NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
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Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

US005936145A

United States Patent [19]

Bradbury

[11] Patent Number: 5,936,145

[45] Date of Patent: Aug. 10, 1999

[54] INBRED CORN PLANT 87DIA4 AND SEEDS THEREOF

[75] Inventor: Peter J. Bradbury, Madison, Wis.

[73] Assignee: DeKalb Genetics Corporation, DeKalb, Ill.

[21] Appl. No.: 09/017,996

[22] Filed: Feb. 3, 1998

Related U.S. Application Data

[60] Provisional application No. 60/037,305, Feb. 5, 1997.

[51] Int. Cl.^{6} A01H 5/00; A01H 4/00; A01H 1/00; C12N 5/04

[52] U.S. Cl. 800/320.1; 800/298; 800/275; 800/271; 800/301; 800/302; 800/303; 435/412; 435/424; 435/430; 435/430.1

[58] Field of Search 800/320.1, 298, 800/275, 271, 303, 274, 302; 435/172.3, 172.1, 412, 424, 430, 430.1

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Primary Examiner—Gary Benzion
Attorney, Agent, or Firm—Arnold, White & Durkee

[57] ABSTRACT

According to the invention, there is provided an inbred corn plant designated 87DIA4. This invention thus relates to the plants, seeds and tissue cultures of the inbred corn plant 87DIA4, and to methods for producing a corn plant produced by crossing the inbred plant 87DIA4 with itself or with another corn plant, such as another inbred. This invention further relates to corn seeds and plants produced by crossing the inbred plant 87DIA4 with another corn plant, such as another inbred, and to crosses with related species. This invention further relates to the inbred and hybrid genetic complements of the inbred corn plant 87DIA4, and also to the RFLP and genetic isozyme typing profiles of inbred corn plant 87DIA4.

39 Claims, No Drawings

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INBRED CORN PLANT 87DIA4 AND SEEDS THEREOF

The present application claims the priority of co-pending U.S. Provisional Patent Application Serial No. 60/037,305, filed Feb. 5, 1997, the entire disclosure of which is incorporated herein by reference without disclaimer.

BACKGROUND OF THE INVENTION

I. Technical Field of the Invention

The present invention relates to the field of corn breeding. In particular, the invention relates to the inbred corn seed and plant designated 87DIA4, and derivatives and tissue cultures of such inbred plant.

II. Description of the Background Art

The goal of field crop breeding is to combine various desirable traits in a single variety/hybrid. Such desirable traits include greater yield, better stalks, better roots, resistance to insecticides, herbicides, pests, and disease, tolerance to heat and drought, reduced time to crop maturity, better agronomic quality, and uniformity in germination times, stand establishment, growth rate, maturity, and fruit size.

Breeding techniques take advantage of a plant's method of pollination. There are two general methods of pollination: a plant self-pollinates if pollen from one flower is transferred to the same or another flower of the same plant. A plant cross-pollinates if pollen comes to it from a flower on a different plant.

Corn plants (*Zea mays L.*) can be bred by both self-pollination and cross-pollination. Both types of pollination involve the corn plant's flowers. Corn has separate male and female flowers on the same plant, located on the tassel and the ear, respectively. Natural pollination occurs in corn when wind blows pollen from the tassels to the silks that protrude from the tops of the ear shoot.

Plants that have been self-pollinated and selected for type over many generations become homozygous at almost all gene loci and produce a uniform population of true breeding progeny, a homozygous plant. A cross between two such homozygous plants produce a uniform population of hybrid plants that are heterozygous for many gene loci. Conversely, a cross of two plants each heterozygous at a number of gene loci produces a population of hybrid plants that differ genetically and are not uniform. The resulting non-uniformity makes performance unpredictable.

The development of uniform corn plant hybrids requires the development of homozygous inbred plants, the crossing of these inbred plants, and the evaluation of the crosses. Pedigree breeding and recurrent selection are examples of breeding methods used to develop inbred plants from breeding populations. Those breeding methods combine the genetic backgrounds from two or more inbred plants or various other broad-based sources into breeding pools from which new inbred plants are developed by selfing and selection of desired phenotypes. The new inbreds are crossed with other inbred plants and the hybrids from these crosses are evaluated to determine which of those have commercial potential.

The pedigree breeding method for single-gene traits involves crossing two genotypes. Each genotype can have one or more desirable characteristics lacking in the other; or, each genotype can complement the other. If the two original parental genotypes do not provide all of the desired characteristics, other genotypes can be included in the breeding population. Superior plants that are the products of these crosses are selfed and selected in successive generations. Each succeeding generation becomes more homogeneous as a result of self-pollination and selection. Typically, this method of breeding involves five or more generations of selfing and selection: $S_1 \rightarrow S_2$; $S_2 \rightarrow S_3$; $S_3 \rightarrow S_4$; $S_4 \rightarrow S_5$, etc. After at least five generations, the inbred plant is considered genetically pure.

Backcrossing can also be used to improve an inbred plant. Backcrossing transfers a specific desirable trait from one inbred or other source to an inbred that lacks that trait. This can be accomplished for example by first crossing a superior inbred (A) (recurrent parent) to a donor inbred (non-recurrent parent), which carries the appropriate gene(s) for the trait in question. The progeny of this cross are then mated back to the superior recurrent parent (A) followed by selection in the resultant progeny for the desired trait to be transferred from the non-recurrent parent. After five or more backcross generations with selection for the desired trait, the progeny are heterozygous for loci controlling the characteristic being transferred, but are like the superior parent for most or almost all other genes. The last backcross generation would be selfed to give pure breeding progeny for the gene(s) being transferred.

A single cross hybrid corn variety is the cross of two inbred plants, each of which has a genotype which complements the genotype of the other. The hybrid progeny of the first generation is designated F_1. Preferred F_1 hybrids are more vigorous than their inbred parents. This hybrid vigor, or heterosis, is manifested in many polygenic traits, including markedly improved higher yields, better stalks, better roots, better uniformity and better insect and disease resistance. In the development of hybrids only the F_1 hybrid plants are sought. An F_1 single cross hybrid is produced when two inbred plants are crossed. A double cross hybrid is produced from four inbred plants crossed in pairs (A×B and C×D) and then the two F_1 hybrids are crossed again (A×B)×(C×D).

The development of a hybrid corn variety involves three steps: (1) the selection of plants from various germplasm pools; (2) the selfing of the selected plants for several generations to produce a series of inbred plants, which, although different from each other, each breed true and are highly uniform; and (3) crossing the selected inbred plants with unrelated inbred plants to produce the hybrid progeny (F_1). During the inbreeding process in corn, the vigor of the plants decreases. Vigor is restored when two unrelated inbred plants are crossed to produce the hybrid progeny (F_1). An important consequence of the homozygosity and homogeneity of the inbred plants is that the hybrid between any two inbreds is always the same. Once the inbreds that give a superior hybrid have been identified, hybrid seed can be reproduced indefinitely as long as the homogeneity of the inbred parents is maintained. Conversely, much of the hybrid vigor exhibited by F_1 hybrids is lost in the next generation (F_2). Consequently, seed from hybrid varieties is not used for planting stock. It is not generally beneficial for farmers to save seed of F_1 hybrids. Rather, farmers purchase F_1 hybrid seed for planting every year.

North American farmers plant over 70 million acres of corn at the present time and there are extensive national and international commercial corn breeding programs. A continuing goal of these corn breeding programs is to develop high-yielding corn hybrids that are based on stable inbred plants that maximize the amount of grain produced and minimize susceptibility to environmental stresses. To accomplish this goal, the corn breeder must select and develop superior inbred parental plants for producing hybrids.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a corn plant designated 87DIA4. Also provided are corn plants having all the physiological and morphological characteristics of corn plant 87DIA4.

The inbred corn plant of the invention may further comprise, or have, a cytoplasmic factor that is capable of conferring male sterility. Parts of the corn plant of the present invention are also provided, such as, e.g., pollen obtained from an inbred plant and an ovule of the inbred plant.

The invention also concerns seed of the corn plant 87DIA4, which has been deposited with the ATCC. The invention thus provides inbred corn seed designated 87DIA4, and having ATCC Accession No. 203192.

The inbred corn seed of the invention may be provided as an essentially homogeneous population of inbred corn seed designated 87DIA4.

Essentially homogeneous populations of inbred seed are those that consist essentially of the particular inbred seed, and are generally purified free from substantial numbers of other seed, so that the inbred seed forms between about 90% and about 100% of the total seed, and preferably, between about 95% and about 100% of the total seed. Most preferably, an essentially homogeneous population of inbred corn seed will contain between about 98.5%, 99%, 99.5% and about 100% of inbred seed, as measured by seed grow outs.

In any event, even if a population of inbred corn seed was found, for some reason, to contain about 50%, or even about 20% or 15% of inbred seed, this would still be distinguished from the small fraction of inbred seed that may be found within a population of hybrid seed, e.g., within a bag of hybrid seed. In such a bag of hybrid seed offered for sale, the Governmental regulations require that the hybrid seed be at least about 95% of the total seed. In the practice of the present invention, the hybrid seed generally forms at least about 97% of the total seed. In the most preferred practice of the invention, the female inbred seed that may be found within a bag of hybrid seed will be about 1% of the total seed, or less, and the male inbred seed that may be found within a bag of hybrid seed will be negligible, i.e., will be on the order of about a maximum of 1 per 100,000, and usually less than this value.

The population of inbred corn seed of the invention is further particularly defined as being essentially free from hybrid seed. The inbred seed population may be separately grown to provide an essentially homogeneous population of inbred corn plant designated 87DIA4.

In another aspect, the present invention provides for single gene converted plants of 87DIA4. The single transferred gene may preferably be a dominant or recessive allele. Preferably, the single transferred gene will confer such traits as male sterility, herbicide resistance, insect resistance, resistance for bacterial, fungal, or viral disease, male fertility, enhanced nutritional quality, and industrial usage. The single gene may be a naturally occurring maize gene or a transgene introduced through genetic engineering techniques.

In another aspect, the present invention provides a tissue culture of regenerable cells of inbred corn plant 87DIA4. The tissue culture will preferably be capable of regenerating plants having the physiological and morphological characteristics of the foregoing inbred corn plant, and of regenerating plants having substantially the same genotype as the foregoing inbred corn plant. Preferably, the regenerable cells in such tissue cultures will be embryos, protoplasts, meristematic cells, callus, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks or stalks. Still further, the present invention provides corn plants regenerated from the tissue cultures of the invention.

In yet another aspect, the present invention provides processes for preparing corn seed or plants, which processes generally comprise crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is the inbred corn plant designated 87DIA4. These processes may be further exemplified as processes for preparing hybrid corn seed or plants, wherein a first inbred corn plant is crossed with a second, distinct inbred corn plant to provide a hybrid that has, as one of its parents, the inbred corn plant 87DIA4.

In a preferred embodiment, crossing comprises planting, in pollinating proximity, seeds of the first and second parent corn plant, and preferably, seeds of a first inbred corn plant and a second, distinct inbred corn plant; cultivating or growing the seeds of said first and second parent corn plants into plants that bear flowers; emasculating the male flowers of the first or second parent corn plant, i.e., treating the flowers so as to prevent pollen production, in order to produce an emasculated parent corn plant; allowing natural cross-pollination to occur between the first and second parent corn plants; and harvesting the seeds from the emasculated parent corn plant. Where desired, the harvested seed is grown to produce a corn plant or hybrid corn plant.

The present invention also provides corn seed and plants produced by a process that comprises crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is the inbred corn plant designated 87DIA4. In one embodiment, corn plants produced by the process are first generation (F_1) hybrid corn plants produced by crossing an inbred in accordance with the invention with another, distinct inbred. The present invention further contemplates seed of an F_1 hybrid corn plant.

In certain exemplary embodiments, the invention provides an F_1 hybrid corn plant and seed thereof, which hybrid corn plant is designated 4033843, having 871DIA4 as one inbred parent.

In yet a further aspect, the invention provides an inbred genetic complement of the corn plant designated 87DIA4. The phrase "genetic complement" is used to refer to the aggregate of nucleotide sequences, the expression of which sequences defines the phenotype of, in the present case, a corn plant, or a cell or tissue of that plant. An inbred genetic complement thus represents the genetic make up of an inbred cell, tissue or plant, and a hybrid genetic complement represents the genetic make up of a hybrid cell, tissue or plant. The invention thus provides corn plant cells that have a genetic complement in accordance with the inbred corn plant cells disclosed herein, and plants, seeds and diploid plants containing such cells.

Plant genetic complements may be assessed by genetic marker profiles, and by the expression of phenotypic traits that are characteristic of the expression of the genetic complement, e.g., isozyme typing profiles. Thus, such corn plant cells may be defined as having an RFLP genetic marker profile in accordance with the profile shown in Table 8, or a genetic isozyme typing profile in accordance with the profile shown in Table 9, or having both an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 8 and 9.

In another aspect, the present invention provides hybrid genetic complements, as represented by corn plant cells, tissues, plants and seeds, formed by the combination of a haploid genetic complement of an inbred corn plant of the invention with a haploid genetic complement of a second corn plant, preferably, another, distinct inbred corn plant. In another aspect, the present invention provides a corn plant regenerated from a tissue culture that comprises a hybrid genetic complement of this invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. DEFINITIONS

Barren Plants: Plants that are barren, i.e., lack an ear with grain, or have an ear with only a few scattered kernels.

Cg: *Colletotrichum graminicola* rating. Rating times 10 is approximately equal to percent total plant infection.

CLN: Corn Lethal Necrosis (combination of Maize (Chlorotic Mottle Virus and Maize Dwarf Mosaic virus) rating: numerical ratings are based on a severity scale where 1=most resistant to 9=susceptible.

Cn: *Corynebacterium nebraskense* rating. Rating times 10 is approximately equal to percent total plant infection.

Cz: *Cercospora zeae-maydis* rating. Rating times 10 is approximately equal to percent total plant infection.

Dgg: *Diatraea grandiosella* girdling rating (values are percent plants girdled and stalk lodged).

Dropped Ears: Ears that have fallen from the plant to the ground.

Dsp: Diabrotica species root ratings (1=least affected to 9=severe pruning).

Ear-Attitude: The attitude or position of the ear at harvest scored as upright, horizontal, or pendant.

Ear-Cob Color: The color of the cob, scored as white, pink, red, or brown.

Ear-Cob Diameter: The average diameter of the cob measured at the midpoint.

Ear-Cob Strength: A measure of mechanical strength of the cobs to breakage, scored as strong or weak.

Ear-Diameter: The average diameter of the ear at its midpoint.

Ear-Dry Husk Color: The color of the husks at harvest scored as buff, red, or purple.

Ear-Fresh Husk The color of the husks 1 to 2 weeks after pollination scored as Color: green, red, or purple.

Ear-Husk Bract: The length of an average husk leaf scored as short, medium, or long.

Ear-Husk Cover: The average distance from the tip of the ear to the tip of the husks. Minimum value no less than zero.

Ear-Husk Opening: An evaluation of husk tightness at harvest scored as tight, intermediate, or open.

Ear-Length: The average length of the ear.

Ear-Number Per The average number of ears per plant. Stalk:

Ear-Shank The average number of internodes on the ear shank. Internodes:

Ear-Shank Length: The average length of the ear shank.

Ear-Shelling Percent: The average of the shelled grain weight divided by the sum of the shelled grain weight and cob weight for a single ear.

Ear-Silk Color: The color of the silk observed 2 to 3 days after silk emergence scored as green-yellow, yellow, pink, red, or purple.

Ear-Taper (Shape): The taper or shape of the ear scored as conical, semi-conical, or cylindrical.

Ear-Weight: The average weight of an ear.

Early Stand: The percent of plants that emerge from the ground as determined in the early spring.

ER: Ear rot rating (values approximate percent ear rotted).

Final Stand Count: The number of plants just prior to harvest.

GDUs to Shed: The number of growing degree units (GDUs) or heat units required for an inbred line or hybrid to have approximately 50 percent of the plants shedding pollen as measured from time of planting. Growing degree units are calculated by the Barger Method, where the heat units for a 24-hour period are calculated as GDUs=[Maximum daily temperature+Minimum daily temperature)/2]-50. The highest maximum daily temperature used is 86 degrees Fahrenheit and the lowest minimum temperature used is 50 degrees Fahrenheit. GDUs to shed is then determined by summing the individual daily values from planting date to the date of 50 percent pollen shed.

GDUs to Silk: The number of growing degree units for an inbred line or hybrid to have approximately 50 percent of the plants with silk emergence as measured from time of planting. Growing degree units are calculated by the same methodology as indicated in the GDUs to shed definition.

Hc2: *Helminthosporium carbonum* race 2 rating. Rating times 10 is approximately equal to percent total plant infection.

Hc3: *Helminthosporium carbonum* race 3 rating. Rating times 10 is approximately equal to percent total plant infection.

Hm: *Helminthosporium maydis* race 0 rating. Rating times 10 is approximately equal to percent total plant infection.

Ht1: *Helminthosporium turcicum* race 1 rating. Rating times 10 is approximately equal to percent total plant infection.

Ht2: *Helminthosporium turcicum* race 2 rating. Rating times 10 is approximately equal to percent total plant infection.

HtG: +=Presence of Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection. −=Absence of a Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection. +/−=Segregation of a Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection.

Kernel-Aleurone The color of the aleurone scored as white, pink, tan, brown, Color: bronze, red, purple, pale purple, colorless, or variegated.

Kernel-Cap Color: The color of the kernel cap observed at dry stage, scored as white, lemon-yellow, yellow or orange.

Kernel-Endosperm The color of the endosperm scored as white, pale yellow, or Color: yellow.

Kernel-Endosperm The type of endosperm scored as normal, waxy, or opaque. Type:

Kernel-Grade: The percent of kernels that are classified as rounds.

Kernel-Length: The average distance from the cap of the kernel to the pedicel.

Kernel-Number Per The average number of kernels in a single row. Row:

Kernel-Pericarp The color of the pericarp scored as colorless, red-white crown, tan, Color: bronze, brown, light red, cherry red, or variegated.

Kernel-Row The direction of the kernel rows on the ear scored as straight, Direction: slightly curved, spiral, or indistinct (scattered).

Kernel-Row Number: The average number of rows of kernels on a single ear.

Kernel-Side Color: The color of the kernel side observed at the dry stage, scored as white, pale yellow, yellow, orange, red, or brown.

Kernel-Thickness: The distance across the narrow side of the kernel.

Kernel-Type: The type of kernel scored as dent, flint, or intermediate.

Kernel-Weight: The average weight of a predetermined number of kernels.

Kernel-Width: The distance across the flat side of the kernel.

Kz: *Kabatiella zeae* rating. Rating times 10 is approximately equal to percent total plant infection.

Leaf-Angle: Angle of the upper leaves to the stalk scored as upright (0 to 30 degrees), intermediate (30 to 60 degrees), or lax (60 to 90 degrees).

Leaf-Color: The color of the leaves 1 to 2 weeks after pollination scored as light green, medium green, dark green, or very dark green.

Leaf-Length: The average length of the primary ear leaf.

Leaf-Longitudinal A rating of the number of longitudinal creases on the leaf surface 1 Creases: to 2 weeks after pollination. Creases are scored as absent, few, or many.

Leaf-Marginal A rating of the waviness of the leaf margin 1 to 2 weeks after Waves: pollination. Rated as none, few, or many.

Leaf-Number: The average number of leaves of a mature plant. Counting begins with the cotyledonary leaf and ends with the flag leaf.

Leaf-Sheath A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks Anthocyanin: after pollination, scored as absent, basal-weak, basal-strong, weak or strong.

Leaf-Sheath A rating of the pubescence of the leaf sheath. Ratings are taken 1 Pubescence: to 2 weeks after pollination and scored as light, medium, or heavy.

Leaf-Width: The average width of the primary ear leaf measured at its widest point.

LSS: Late season standability (values times 10 approximate percent plants lodged in disease evaluation plots).

Moisture: The moisture of the grain at harvest.

On1: *Ostrinia nubilalis* 1st brood rating (1=resistant to 9=susceptible).

On2: *Ostrinia nubilalis* 2nd brood rating (1=resistant to 9=susceptible).

Relative Maturity: A maturity rating based on regression analysis. The regression analysis is developed by utilizing check hybrids and their previously established day rating versus actual harvest moistures. Harvest moisture on the hybrid in question is determined and that moisture value is inserted into the regression equation to yield a relative maturity.

Root Lodging: Root lodging is the percentage of plants that root lodge. A plant is counted as root lodged if a portion of the plant leans from the vertical axis by approximately 30 degrees or more.

Seedling Color: Color of leaves at the 6 to 8 leaf stage.

Seedling Height: Plant height at the 6 to 8 leaf stage.

Seedling Vigor: A visual rating of the amount of vegetative growth on a 1 to 9 scale, where 1 equals best. The score is taken when the average entry in a trial is at the fifth leaf stage.

Selection Index: The selection index gives a single measure of hybrid's worth based on information from multiple traits. One of the traits that is almost always included is yield. Traits may be weighted according to the level of importance assigned to them.

Sr: *Sphacelotheca reiliana* rating is actual percent infection.

Stalk-Anthocyanin: A rating of the amount of anthocyanin pigmentation in the stalk. The stalk is rated 1 to 2 weeks after pollination as absent, basal-weak, basal-strong, weak, or strong.

Stalk-Brace Root The color of the brace roots observed 1 to 2 weeks after pollination Color: as green, red, or purple.

Stalk-Diameter: The average diameter of the lowest visible internode of the stalk.

Stalk-Ear Height: The average height of the ear measured from the ground to the point of attachment of the ear shank of the top developed ear to the stalk.

Stalk-Internode The direction of the stalk internode observed after pollination as Direction: straight or zigzag.

Stalk-Internode The average length of the internode above the primary ear. Length:

Stalk Lodging: The percentage of plants that did stalk lodge. Plants are counted as stalk lodged if the plant is broken over or off below the ear.

Stalk-Nodes With The average number of nodes having brace roots per plant. Brace Roots:

Stalk-Plant Height: The average height of the plant as measured from the soil to the tip of the tassel.

Stalk-Tillers: The percent of plants that have tillers. A tiller is defined as a secondary shoot that has developed as a tassel capable of shedding pollen.

Staygreen: Staygreen is a measure of general plant health near the time of black layer formation (physiological maturity). It is usually recorded at the time the ear husks of most entries within a trial have turned a mature color. Scoring is on a 1 to 9 basis where 1 equals best.

STR: Stalk rot rating (values represent severity rating of 1=25 percent of inoculated internode rotted to 9=entire stalk rotted and collapsed).

SVC: Southeastern Virus Complex combination of Maize Chlorotic Dwarf Virus and Maize Dwarf Mosaic Virus) rating; numerical ratings are based on a severity scale where 1=most resistant to 9=susceptible (1988 reactions are largely Maize Dwarf Mosaic Virus reactions).

Tassel-Anther Color: The color of the anthers at 50 percent pollen shed scored as green-yellow, yellow, pink, red, or purple.

Tassel-Attitude: The attitude of the tassel after pollination scored as open or compact.

Tassel-Branch Angle: The angle of an average tassel branch to the main stem of the tassel scored as upright (less than 30 degrees), intermediate (30 to 45 degrees), or lax (greater than 45 degrees).

Tassel-Branch The average number of primary tassel branches. Number:

Tassel-Glume Band: The closed anthocyanin band at the base of the glume scored as present or absent.

Tassel-Glume Color: The color of the glumes at 50 percent shed scored as green, red, or purple.

Tassel-Length: The length of the tassel measured from the base of the bottom tassel branch to the tassel tip.

Tassel-Peduncle The average length of the tassel peduncle, measured from the base Length: of the flag leaf to the base of the bottom tassel branch.

Tassel-Pollen Shed: A visual rating of pollen shed determined by tapping the tassel and observing the pollen flow of approximately five plants per entry. Rated on a 1 to 9 scale where 9=sterile, 1=most pollen.

Tassel-Spike Length: The length of the spike measured from the base of the top tassel branch to the tassel tip.

Test Weight: The measure of the weight of the grain in pounds for a given volume (bushel) adjusted to 15.5 percent moisture.

Yield: Yield of grain at harvest adjusted to 15.5 percents moisture.

II. OTHER DEFINITIONS

Allele is any of one or more alternative forms of a gene, all of which alleles relate to one trait or characteristic. In a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes.

Backcrossing is a process in which a breeder repeatedly crosses hybrid progeny back to one of the parents, for example, a first generation hybrid (F_1) with one of the parental genotypes of the F1 hybrid.

Chromatography is a technique wherein a mixture of dissolved substances are bound to a solid support followed by passing a column of fluid across the solid support and varying the composition of the fluid. The components of the mixture are separated by selective elution.

Crossing refers to the mating of two parent plants.

Cross-pollination refers to fertilization by the union of two gametes from different plants.

Diploid refers to a cell or organism having two sets of chromosomes.

Electrophoresis is a process by which particles suspended in a fluid are moved under the action of an electrical field, and thereby separated according to their charge and molecular weight. This method of separation is well known to those skilled in the art and is typically applied to separating various forms of enzymes and of DNA fragments produced by restriction endonucleases.

Emasculate refers to the removal of plant male sex organs.

Enzymes are organic catalysts that can exist in various forms called isozymes.

F_1 Hybrid refers to the first generation progeny of the cross of two plants.

Genetic Complement refers to an aggregate of nucleotide sequences, the expression of which sequences defines the phenotype in corn plants, or components of plants including cells or tissue.

Genotype refers to the genetic constitution of a cell or organism.

Haploid refers to a cell or organism having one set of the two sets of chromosomes in a diploid.

Isozymes are one of a number of enzymes which catalyze the same reaction(s) but differ from each other, e.g., in primary structure and/or electrophoretic mobility. The differences between isozymes are under single gene, codominant control. Consequently, electrophoretic separation to produce band patterns can be equated to different alleles at the, DNA level. Structural differences that do not alter charge cannot be detected by this method.

Isozyme typing profile refers to a profile of band patterns of isozymes separated by electrophoresis that can be equated to different alleles at the DNA level.

Linkage refers to a phenomenon wherein alleles on the same chromosome tend to segregate together more often than expected by chance if their transmission was independent.

Marker is a readily detectable phenotype, preferably inherited in codominant fashion (both alleles at a locus in a diploid heterozygote are readily detectable), with no environmental variance component, i.e., heritability of 1.

87DIA4 refers to the corn plant from which seeds having ATCC Accession No. 203192 were obtained, as well as plants grown from those seeds.

Phenotype refers to the detectable characteristics of a cell or organism, which characteristics are the manifestation of gene expression.

Quantitative Trait Loci (QTL) refer to genetic loci that control to some degree numerically representable traits that are usually continuously distributed.

Regeneration refers to the development of a plant from tissue culture.

RFLP genetic marker profile refers to a profile of band patterns of DNA fragment lengths typically separated by agarose gel electrophoresis, after restriction endonuclease digestion of DNA.

Self-pollination refers to the transfer of pollen from the anther to the stigma of the same plant.

Single Gene Converted (Conversion) Plant refers to plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single gene transferred into the inbred via the backcrossing technique.

Tissue Culture refers to a composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

III. INBRED CORN PLANT 87DIA4

In accordance with one aspect of the present invention, there is provided a novel inbred corn plant, designated 87DIA4. Inbred corn plant 87DIA4 is a yellow, dent corn inbred that can be compared to inbred corn plants 2FACC, 3AZA1, and AQA3, all of which are proprietary inbreds of DEKALB Genetics Corporation. 87DIA4 differs significantly (at the 1%, 5%, or 10% level) from these inbred lines in several aspects (Table 1, Table 2, and Table 3).

TABLE 1

COMPARISON OF 87DIA4 WITH 2FACC

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
2FACC	0.4	0.6	29.5	62.0	23.9	67.3	1.3	1482.6	1481.5	5.8	77.4
DIFF	-0.1	-0.5	-5.2	0.4	-6.1	-10.1	-1.1	-119.5	-124.4	2.4	-12.3
# LOC	15	13	8	15	14	8	14	8	8	13	13
P VALUE	0.88	0.65	0.00**	0.84	0.00**	0.00**	0.40	0.00**	0.00**	0.36	0.01*

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

TABLE 2

COMPARISON OF 87DIA4 WITH 3AZA1

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
3AZA1	1.1	1.6	23.6	62.0	15.9	58.4	0.1	1322.6	1321.2	8.4	41.1
DIFF	-0.8	-1.5	0.7	0.4	1.9	-1.4	0.1	40.5	35.9	-0.2	24.0
# LOC	15	13	8	15	14	8	14	8	8	13	13
P VALUE	0.41	0.19	0.66	0.84	0.13	0.48	0.94	0.00**	0.03*	0.94	0.00**

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

TABLE 3

COMPARISON OF 87DIA4 WITH AQA3

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
AQA3	0.4	0.5	25.9	62.7	14.4	58.1	1.0	1356.2	1348.6	15.2	35.7
DIFF	-0.1	-0.4	-1.6	-0.3	3.3	-1.1	-0.8	6.9	8.5	-7.0	29.4

TABLE 3-continued

COMPARISON OF 87DIA4 WITH AQA3												
INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
# LOC	15	13	8	15	14	8	14	8	8	10	13
P VALUE	0.86	0.72	0.34	0.86	0.00**	0.58	0.56	0.64	0.61	0.01*	0.00**

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

A. ORIGIN AND BREEDING HISTORY

Inbred plant 87DIA4 was derived from the cross between a line derived from 2FACC and 3AZA1.

87DIA4 shows uniformity and stability within the limits of environmental influence for the traits described hereinafter in Table 4. 87DIA4 has been self-pollinated and ear-rowed a sufficient number of generations with careful attention paid to uniformity of plant type to ensure homozygosity and phenotypic stability. No variant traits have been observed or are expected in 87DIA4.

A deposit of 2500 seeds of plant designated 87DIA4 has been made with the American Type Culture Collection (ATCC), Rockville Pike, Bethesda, Md. on Sep. 11, 1998. Those deposited seeds have been assigned Accession No. 203192. The deposit was made in accordance with the terms and provisions of the Budapest Treaty relating to deposit of microorganisms and is made for a term of at least thirty (30) years and at least five (05) years after the most recent request for the furnishing of a sample of the deposit was received by the depository, or for the effective term of the patent, whichever is longer, and will be replaced if it becomes non-viable during that period.

Inbred corn plants can be reproduced by planting such inbred seeds, growing the resulting corn plants under self-pollinating or sib-pollinating conditions with adequate isolation using standard techniques well known to an artisan skilled in the agricultural arts. Seeds can be harvested from such a plant using standard, well known procedures.

The origin and breeding history of inbred plant 87DIA4 can be summarized as follows:

Summer 1988 The cross 2FACC and AQA3 was made. Both inbreds are proprietary to DEKALB Genetics Corporation.

Winter 1988 S0 seed was grown (nursery row 67-51).

Summer 1989 S1 seed was grown (nursery rows 4-25 to 4-38).

Winter 1989 S2 seed was grown ear-to-row (nursery row 649-62).

Summer 1990 S3 seed was grown ear-to-row (nursery row 130-15).

Winter 1990 S4 seed was grown ear-to-row (nursery row C23-23).

Summer 1991 S5 seed was grown ear-to-row (nursery row 222-67).

Summer 1992 S6 seed was grown ear-to-row (nursery row 418-56).

Summer 1993 S7 seed was grown ear-to-row (nursery rows 346-32 to 346-39). Seed from all rows was bulked to form 87DIA4.

B. PHENOTYPIC DESCRIPTION

In accordance with another aspect of the present invention, there is provided a corn plant having the physiological and morphological characteristics of corn plant 87DIA4. A description of the physiological and morphological characteristics of corn plant 87DIA114 is presented in Table 4.

TABLE 4

MORPHOLOGICAL TRAITS FOR THE 87DIA4 PHENOTYPE				
CHARACTERISTIC	87DIA4	2FACC	3AZA1	AQA3
1. STALK				
Diameter (width) cm	1.9	2.2	2.0	2.2
Anthocyanin	Absent	Absent	Absent	Absent
Nodes with Brace Roots	1.4	1.9	2.0	1.5
Brace Root Color	Red	Purple	—	Green
Internode Direction	Straight	Straight	Straight	Straight

TABLE 4-continued

MORPHOLOGICAL TRAITS FOR THE 87DIA4 PHENOTYPE				
CHARACTERISTIC	87DIA4	2FACC	3AZA1	AQA3
Internode Length cm.	10.2	12.7	14.0	11.3
2. LEAF				
Color	Med Green	Med Green	Med Green	Med Green
Length cm.	68.0	71.1	66.2	67.9
Width cm.	10.0	8.7	7.9	8.3
Sheath Anthocyanin	Weak	Weak	Weak	Absent
Sheath Pubescence	Medium	Light	Medium	Medium
Marginal Waves	Few	Few	Few	Few
Longitudinal Creases	Absent	Absent	—	Few
3. TASSEL				
Attitude	Compact	Compact	—	Open
Length cm.	29.5	26.7	33.0	33.0
Spike Length cm.	19.5	19.1	24.4	23.1
Peduncle Length cm.	2.9	5.2	3.6	3.6
Branch Number	4.5	7.7	3.8	5.5
Anther Color	Red	Pink	Tan	Grn-Yellow
Glume Color	Green	Green	Green	Green
Glume Band	Absent	Absent	Absent	Absent
4. EAR				
Silk Color	Pink	Tan	Grn-Yellow	Grn-Yellow
Number Per Stalk	1.1	1.1	1.6	1.4
Position (attitude)	Upright	Upright	Pendant	Upright
Length cm.	15.6	13.9	16.4	16.3
Shape	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Diameter cm.	3.8	4.2	3.4	3.6
Weight gm.	99.1	116.3	89.8	93.1
Shank Length cm.	16.5	14.8	20.7	14.9
Husk Bract	Short	Short	Short	Short
Husk Cover cm.	3.4	6.6	1.9	3.2
Husk Opening	Tight	Intermediate	—	Intermediate
Husk Color Fresh	Green	Lt Green	Green	Lt Green
Husk Color Dry	Buff	Buff	Buff	Buff
Cob Diameter cm.	2.5	2.6	1.7	2.1
Cob Color	Red	Red	Red	Red
Shelling Percent	85.1	81.4	85.8	85.0
5. KERNEL				
Row Number	14.0	14.7	12.3	15.1
Number Per row	31.4	25.5	32.2	33.2
Row Direction	Curved	Curved	Curved	Curved
Type	Dent	Dent	Dent	Dent
Cap Color	Yellow	Yellow	Yellow	Lemon Yellow
Side Color	Yellow	Deep Yellow	Orange	Orange
Length (depth) mm.	10.2	10.7	9.2	9.6
Width mm.	8.1	8.1	7.3	7.1
Thickness	4.3	4.3	4.2	3.8
Weight of 1000K gm.	281.5	280.7	223.8	173.7
Endosperm Type	Normal	Normal	Normal	Normal
Endosperm Color	Yellow	Yellow	Yellow	Yellow

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

IV. ADDITIONAL INBRED CORN PLANTS

The inbred corn plant 171KI3 has been employed with the corn plant of the present invention in order to produce an exemplary hybrid. A description of the physiological and morphological characteristics of this corn plant is presented herein at Table 5. Additional information for this inbred corn plant is presented in co-pending U.S. patent application Ser. No. 08/795,403, filed Feb. 5, 1997, the disclosure of which application is specifically incorporated herein by reference.

TABLE 5

MORPHOLOGICAL TRAITS FOR THE 171KI3 PHENOTYPE				
CHARACTERISTIC	171KI3	01CSI2	01IBH2	3IIH6
1. STALK				
Diameter (width) cm.	2.2	2.4	2.1	2.3

TABLE 5-continued

MORPHOLOGICAL TRAITS FOR THE 171KI3 PHENOTYPE

CHARACTERISTIC	171KI3	01CSI2	01IBH2	3IIH6
Anthocyanin	Absent	Absent	Absent	Absent
Nodes With Brace	0.9	1.8	1.1	0.7
Roots				
Brace Root Color	Green	Green	Green	—
Internode Direction	Straight	Straight	Straight	Straight
Internode Length	15.9	12.8	14.4	13.1
cm.				
2. LEAF				
Color	Med Green	—	Med Green	Med Green
Width cm.	9.7	8.9	8.9	8.0
Marginal Waves	Few	Few	Few	Few
3. TASSEL				
Length cm.	42.6	31.2	33.6	35.3
Spike Length cm.	22.9	23.2	23.1	25.2
Peduncle Length	9.6	3.9	8.2	7.6
cm.				
Branch Number	9.1	7.4	7.8	12.9
Anther Color	Purple	Grn-Yellow	Grn-Yellow	—
Glume Color	Purple	Green	Green	—
Glume Band	Present	Absent	Absent	Absent
4. EAR	Pink	Pink	—	Red
Silk Color	1.0	1.0	1.0	1.4
Number Per Stalk	Upright	—	—	Upright
Position (attitude)	14.6	16.0	14.6	15.6
Length cm.	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Shape	4.0	3.8	4.0	3.9
Diameter cm.	104.9	100.6	103.2	107.6
Weight gm.	10.3	14.1	10.1	9.6
Shank Length cm.	Short	Short	Short	Short
Husk Bract	6.4	2.5	4.4	3.7
Husk Cover cm.	Green	Green	Green	Green
Husk Color Fresh	Buff	Buff	Buff	Buff
Husk Color Dry	2.3	2.4	2.3	2.1
Cob Diameter cm.	Red	—	Red	Red
Cob Color	87.7	80.6	89.0	83.3
Shelling Percent				
5. KERNEL	14.6	14.8	16.3	15.3
Row Number	32.1	27.1	29.3	29.7
Number Per Row	Curved	Curved	Curved	Curved
Row Direction	Dent	—	Dent	—
Type	Yellow	Yellow	Yellow	Yellow
Cap Color	Deep Yellow	—	Orange	
Side Color	11.1	9.4	10.9	10.3
Length (depth) mm.	7.8	8.0	7.4	7.8
Width mm.	3.9	5.2	4.4	4.2
Thickness	269.0	252.4	233.0	247.8
Weight of 1000K	Normal	Normal	Normal	Normal
gm.				
Endosperm Type	Yellow	Yellow	Yellow	Yellow
Endosperm Color				

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

V. SINGLE GENE CONVERSIONS

When the term inbred corn plant is used in the context of the present invention, this also includes any single gene conversions of that inbred. The term single gene converted plant as used herein refers to those corn plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single gene transferred into the inbred via the backcrossing technique. Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the inbred. The term backcrossing as used herein refers to the repeated crossing of a hybrid progeny back to one of the parental corn plants for that inbred. The parental corn plant which contributes the gene for the desired characteristic is termed the nonrecurrent or donor parent. This terminology refers to the fact that the nonrecurrent parent is used one time in the backcross protocol and therefore does not recur. The parental corn plant to which the gene or genes from the nonrecurrent parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol (Poehlman & Sleper, 1994; Fehr, 1987). In a typical backcross protocol, the original inbred of interest (recurrent parent) is crossed to a second inbred (nonrecurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a corn plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the

converted plant, in addition to the single transferred gene from the nonrecurrent parent.

The selection of a suitable recurrent parent is an important step for a successful backcrossing procedure. The goal of a backcross protocol is to alter or substitute a single trait or characteristic in the original inbred. To accomplish this, a single gene of the recurrent inbred is modified or substituted with the desired gene from the nonrecurrent parent, while retaining essentially all of the rest of the desired genetic, and therefore the desired physiological and morphological, constitution of the original inbred. The choice of the particular nonrecurrent parent will depend on the purpose of the backcross, one of the major purposes is to add some commercially desirable, agronomically important trait to the plant. The exact backcrossing protocol will depend on the characteristic or trait being altered to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele may also be transferred. In this instance it may be necessary to introduce a test of the progeny to determine if the desired characteristic has been successfully transferred.

Many single gene traits have been identified that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques. Single gene traits may or may not be transgenic, examples of these traits include but are not limited to, male sterility, waxy starch, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, male fertility, enhanced nutritional quality, industrial usage, yield stability and yield enhancement. These genes are generally inherited through the nucleus. Some known exceptions to this are the genes for male sterility, some of which are inherited cytoplasmically, but still act as single gene traits. Several of these single gene traits are described in U.S. Ser. No. 07/113,561, filed Aug. 25, 1993, the disclosure of which is specifically hereby incorporated by reference.

Direct selection may be applied where the single gene acts as a dominant trait. An example might be the herbicide resistance trait. For this selection process, the progeny of the initial cross are sprayed with the herbicide prior to the backcrossing. The spraying eliminates any plants which do not have the desired herbicide resistance characteristic, and only those plants which have the herbicide resistance gene are used in the subsequent backcross. This process is then repeated for all additional backcross generations.

The waxy characteristic is an example of a recessive trait. In this example, the progeny resulting from the first backcross generation (BC1) must be grown and selfed. A test is then run on the selfed seed from the BC1 plant to determine which BC1 plants carried the recessive gene for the waxy trait. In other recessive traits, additional progeny testing, for example growing additional generations such as the BC1S1 may be required to determine which plants carry the recessive gene.

VI. ORIGIN AND BREEDING HISTORY OF AN EXEMPLARY SINGLE GENE CONVERTED PLANT

85DGD1 MLms is a single gene conversion of 85DGD1 to cytoplasmic male sterility. 85DGD1 MLms was derived using backcross methods. 85DGD1 (a proprietary inbred of DEKALB Genetics Corporation) was used as the recurrent parent and MLms, a germplasm source carrying ML cytoplasmic sterility, was used as the nonrecurrent parent. The breeding history of the single gene converted inbred 85DGD1 MLms can be summarized as follows:

Hawaii Nurseries Planting Date Apr. 2, 1992 Made up S-O: Female row 585 male row 500

Hawaii Nurseries Planting Date Jul. 15, 1992 S-O was grown and plants were backcrossed times 85DGD1 (rows 444' 443)

Hawaii Nurseries Planting Date Bulked seed of the BC1 was grown and Nov. 18, 1992 backcrossed times 85DGD1 (rows V3-27' V3-26)

Hawaii Nurseries Planting Date Apr. 2, 1993 Bulked seed of the BC2 was gown and backcrossed times 85DGD1 (rows 37' 36)

Hawaii Nurseries Planting Date Jul. 14, 1993 Bulked seed of the BC3 was grown and backcrossed times 85DGD1 (rows 99' 98)

Hawaii Nurseries Planting Date Bulked seed of BC4 was grown and backcrossed Oct. 28, 1993 times 85DGD1 (rows KS-63' KS-62)

Summer 1994 A single ear of the BC5 was grown and backcrossed times 85DGD1 (MC94-822' MC94-822-7)

Winter 1994 Bulked seed of the BC6 was grown and backcrossed times 85DGD1 (3Q-1' 3Q-2)

Summer 1995 Seed of the BC7 was bulked and named 85DGD1 MLms.

VII. TISSUE CULTURE AND IN VITRO REGENERATION OF CORN PLANTS

A further aspect of the invention relates to tissue culture of corn plants designated 87DIA4. As used herein, the term "tissue culture" indicates a composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant. Exemplary types of tissue cultures are protoplasts, calli, plant clumps, and plant cells that are intact in plants or parts of plants, such as embryos, pollen, flowers, kernels, ears, cobs, leaves, husks, stalks, roots, root tips, anthers, silk and the like. In a preferred embodiment, tissue culture is embryos, protoplast, meristematic cells, pollen, leaves or anthers. Means for preparing and maintaining plant tissue culture are well known in the art. By way of example, a tissue culture comprising organs such as tassels or anthers, has been used to produce regenerated plants. (See, U.S. patent applications Ser. No. 07/992,637, filed Dec. 18, 1992 and 07/995,938, filed Dec. 21, 1992, now issued as U.S. Pat. No. 5,322,789, issued Jun. 21, 1994, the disclosures of which are incorporated herein by reference).

VIII. TASSEL/ANTHER CULTURE

Tassels contain anthers which in turn enclose microspores. Microspores develop into pollen. For anther/microspore culture, if tassels are the plant composition., they are preferably selected at a stage when the microspores are uninucleate, that is, include only one, rather than 2 or 3 nuclei. Methods to determine the correct stage are well known to those killed in the art and include mitramycin fluorescent staining (Pace et al., 1987), trypan blue (preferred) and acetocarmine squashing. The mid-uninucleate microspore stage has been found to be the developmental stage most responsive to the subsequent methods disclosed to ultimately produce plants.

Although microspore-containing plant organs such as tassels can generally be pretreated at any cold temperature below about 25° C., a range of 4 to 25° C. is preferred, and a range of 8 to 14° C. is particularly preferred. Although other temperatures yield embryoids and regenerated plants, cold temperatures produce optimum response rates compared to pretreatment at temperatures outside the preferred range. Response rate is measured as either the number of embryoids or the number of regenerated plants per number of microspores initiated in culture.

Although not required, when tassels are employed as the plant organ, it is generally preferred to sterilize their surface.

Following surface sterilization of the tassels, for example, with a solution of calcium hypochloride, the anthers are removed from about 70 to 150 spikelets (small portions of the tassels) and placed in a preculture or pretreatment medium. Larger or smaller amounts can be used depending on the number of anthers.

When one elects to employ tassels directly, tassels are preferably pretreated at a cold temperature for a predefined time, preferably at 10° C. for about 4 days. After pretreatment of a whole tassel at a cold temperature, dissected anthers are further pretreated in an environment that diverts microspores from their developmental pathway. The function of the preculture medium is to switch the developmental program from one of pollen development that of embryoid/callus development. An embodiment of such an environment in the form of a preculture medium includes a sugar alcohol, for example mannitol or sorbitol, inositol or the like. An exemplary synergistic combination is the use of mannitol at a temperature of about 10° C. for a period ranging from about 10 to 14 days. In a preferred embodiment, 3 ml of 0.3 M mannitol combined with 50 mg/l of ascorbic acid, silver nitrate and colchicine is used for incubation of anthers at 10° C. for between 10 and 14 days. Another embodiment is to substitute sorbitol for mannitol. The colchicine produces chromosome doubling at this early stage. The chromosome doubling agent is preferably only present at the preculture stage.

It is believed that the mannitol or other similar carbon structure or environmental stress induces starvation and functions to force microspores to focus their energies on entering developmental stages. The cells are unable to use, for example, mannitol as a carbon source at this stage. It is believed that these treatments confuse the cells causing them to develop as embryoids and plants from microspores. Dramatic increases in development from these haploid cells, as high as 25 embryoids in 10^4 microspores, have resulted from using these methods.

In embodiments where microspores are obtained from anthers, microspores can be released from the anthers into an isolation medium following the mannitol preculture step. One method of release is by disruption of the anthers, for example, by chopping the anthers into pieces with a sharp instrument, such as a razor blade, scalpel or Waring blender. The resulting mixture of released microspores, anther fragments and isolation medium are then passed through a filter to separate microspores from anther wall fragments. An embodiment of a filter is a mesh, more specifically, a nylon mesh of about 112 mm pore size. The filtrate which results from filtering the microspore-containing solution is preferably relatively free of anther fragments, cell walls and other debris.

In a preferred embodiment, isolation of microspores is accomplished at a temperature below about 25° C. and, preferably at a temperature of less than about 15° C. Preferably, the isolation media, dispersing tool (e.g., razor blade) funnels, centrifuge tubes and dispersing container (e.g., petri dish) are all maintained at the reduced temperature during isolation. The use of a precooled dispersing tool to isolate maize microspores has been reported (Gaillard et al., 1991).

Where appropriate and desired, the anther filtrate is then washed several times in isolation medium. The purpose of the washing and centrifugation is to eliminate any toxic compounds which are contained in the non-microspore part of the filtrate and are created by the chopping process. The centrifugation is usually done at decreasing spin speeds, for example, 1000, 750, and finally 500 rpms.

The result of the foregoing steps is the preparation of a relatively pure tissue culture suspension of microspores that are relatively free of debris and anther remnants.

To isolate microspores, an isolation media is preferred. An isolation media is used to separate microspores from the anther walls while maintaining their viability and embryogenic potential. An illustrative embodiment of an isolation media includes a 6 percent sucrose or maltose solution combined with an antioxidant such as 50 mg/l of ascorbic acid, 0.1 mg/l biotin and 400 mg/l of proline, combined with 10 mg/l of nicotinic acid and 0.5 mg/l $AgNO_3$. In another embodiment, the biotin and proline are omitted.

An isolation media preferably has a higher antioxidant level where used to isolate microspores from a donor plant (a plant from which a plant composition containing a microspore is obtained) that is field grown in contrast to greenhouse grown. A preferred level of ascorbic acid in an isolation medium is from about 50 mg/l to about 125 mg/l and, more preferably from about 50 mg/l to about 100 mg/l.

One can find particular benefit in employing a support for the microspores during culturing and subculturing. Any support that maintains the cells near the surface can be used. The microspore suspension is layered onto a support, for example by pipetting. There are several types of supports which are suitable and are within the scope of the invention. An illustrative embodiment of a solid support is a TRANSWELL® culture dish. Another embodiment of a solid support for development of the microspores is a bilayer plate wherein liquid media is on top of a solid base. Other embodiments include a mesh or a millipore filter. Preferably, a solid support is a nylon mesh in the shape of a raft. A raft is defined as an approximately circular support material which is capable of floating slightly above the bottom of a tissue culture vessel, for example, a petri dish, of about a 60 or 100 mm size, although any other laboratory tissue culture vessel will suffice. In an illustrative embodiment, a raft is about 55 mm in diameter.

Culturing isolated microspores on a solid support, for example, on a 10 mm pore nylon raft floating on 2.2 ml of medium in a 60 mm petri dish, prevents microspores from sinking into the liquid medium and thus avoiding low oxygen tension. These types of cell supports enable the serial transfer of the nylon raft with its associated microspore/embryoids ultimately to full strength medium containing activated charcoal and solidified with, for example, GELRITE™ (solidifying agent). The charcoal is believed to absorb toxic wastes and intermediaries. The solid medium allows embryoids to mature.

The liquid medium passes through the mesh while the microspores are retained and supported at the medium-air interface. The surface tension of the liquid medium in the petri dish causes the raft to float. The liquid is able to pass through the mesh: consequently, the microspores stay on top. The mesh remains on top of the total volume of liquid medium. An advantage of the raft is to permit diffusion of nutrients to the microspores. Use of a raft also permits transfer of the microspores from dish to dish during subsequent subculture with minimal loss, disruption or disturbance of the induced embryoids that are developing. The rafts represent an advantage over the multi-welled TRANSWELL® plates, which are commercially available from COSTAR, in that the commercial plates are expensive. Another disadvantage of these plates is that to achieve the serial transfer of microspores to subsequent media, the membrane support with cells must be peeled off the insert in the wells. This procedure does not produce as good a yield nor as efficient transfers, as when a mesh is used as a vehicle for cell transfer.

The culture vessels can be further defined as either (1) a bilayer 60 mm petri plate wherein the bottom 2 ml of medium are solidified with 0.7 percent agarose, overlaid with 1 mm of liquid containing the microspores; (2) a nylon mesh raft wherein a wafer of nylon is floated on 1.2 ml of medium and 1 ml of isolated microspores is pipetted on top; or (3) TRANSWELL® plates wherein isolated microspores are pipetted onto membrane inserts which support the microspores at the surface of 2 ml of medium.

After the microspores have been isolated, they are cultured in a low strength anther culture medium until about the 50 cell stage when they are subcultured onto an embryoid/callus maturation medium. Medium is defined at this stage as any combination of nutrients that permit the microspores to develop into embryoids or callus. Many examples of suitable embryoid/callus promoting media are well known to those skilled in the art. These media will typically comprise mineral salts, a carbon source, vitamins, growth regulations. A solidifying agent is optional. A preferred embodiment of such a media is referred to by the inventor as the "D medium" which typically includes 6N1 salts, $AgNO_3$ and sucrose or maltose.

In an illustrative embodiment, 1 ml of isolated microspores are pipetted onto a 10 mm nylon raft and the raft is floated on 1.2 ml of medium "D", containing sucrose or, preferably maltose. Both calli and embryoids can develop. Calli are undifferentiated aggregates of cells. Type I is a relatively compact, organized and slow growing callus. Type II is a soft, friable and fast-growing one. Embryoids are aggregates exhibiting some embryo-like structures. The embryoids are preferred for subsequent steps to regenerating plants. Culture medium "D" is an embodiment of medium that follows the isolation medium and replaces it. Medium "D" promotes growth to an embryoid/callus. This medium comprises 6N1 salts at ⅛ the strength of a basic stock solution, (major components) and minor components, plus 12 percent sucrose or, preferably 12 percent maltose, 0.1 mg/l B1, 0.5 mg/l nicotinic acid, 400 mg/l proline and 0.5 mg/l silver nitrate. Silver nitrate is believed to act as an inhibitor to the action of ethylene. Multi-cellular structures of approximately 50 cells each generally arise during a period of 12 days to 3 weeks. Serial transfer after a two week incubation period is preferred.

After the petri dish has been incubated for an appropriate period of time, preferably two weeks, in the dark at a predefined temperature, a raft bearing the dividing microspores is transferred serially to solid based media which promotes embryo maturation. In an illustrative embodiment, the incubation temperature is 30° C. and the mesh raft supporting the embryoids is transferred to a 100 mm petri dish containing the 6N1-TGR-4P medium, an "anther culture medium." This medium contains 6N1 salts, supplemented with 0.1 mg/l TIBA, 12 percent sugar (sucrose, maltose or a combination thereof), 0.5 percent activated charcoal, 400 mg/l proline, 0.5 mg/l B, 0.5 mg/l nicotinic acid, and 0.2 percent GELRITE™ (solidifying agent) and is capable of promoting the maturation of the embryoids. Higher quality embryoids, that is, embryoids which exhibit more organized development, such as better shoot meristem formation without precocious germination were typically obtained with the transfer to full strength medium compared to those resulting from continuous culture using only, for example, the isolated microspore culture (IMC) Medium "D." The maturation process permits the pollen embryoids to develop further in route toward the eventual regeneration of plants. Serial transfer occurs to full strength solidified 6N1 medium using either the nylon raft, the TRANSWELL® membrane or bilayer plates, each one requiring the movement of developing embryoids to permit further development into physiologically more mature structures.

In an especially preferred embodiment, microspores are isolated in an isolation media comprising about 6 percent maltose, cultured for about two weeks in an embryoid/calli induction medium comprising about 12 percent maltose and then transferred to a solid medium comprising about 12 percent sucrose.

At the point of transfer of the raft after about two weeks incubation, embryoids exist on a nylon support. The purpose of transferring the raft with the embryoids to a solidified medium after the incubation is to facilitate embryo maturation. Mature embryoids at this point are selected by visual inspection indicated by zygotic embryo-like dimensions and structures and are transferred to the shoot initiation medium. It is preferred that shoots develop before roots, or that shoots and roots develop concurrently. If roots develop before shoots, plant regeneration can be impaired. To produce solidified media, the bottom of a petri dish of approximately 100 mm is covered with about 30 ml of 0.2 percent GELRITE™ (solidifying agent) solidified medium. A sequence of regeneration media are used for whole plant formation from the embryoids.

During the regeneration process, individual embryoids are induced to form plantlets. The number of different media in the sequence can vary depending on the specific protocol used. Finally, a rooting medium is used as a prelude to transplanting to soil. When plantlets reach a height of about 5 cm, they are then transferred to pots for further growth into flowering plants in a greenhouse by methods well known to those skilled in the art.

Plants have been produced from isolated microspore cultures by methods disclosed herein, including self-pollinated plants. The rate of embryoid induction was much higher with the synergistic preculture treatment consisting of a combination of stress factors, including a carbon source which can be capable of inducing starvation, a cold temperature and colchicine, than has previously been reported. An illustrative embodiment of the synergistic combination of treatments leading to the dramatically improved response rate compared to prior methods, is a temperature of about 10° C., mannitol as a carbon source, and 0.05 percent colchicine.

The inclusion of ascorbic acid, an anti-oxidant, in the isolation medium is preferred for maintaining good microspore viability. However, there seems to be no advantage to including mineral salts in the isolation medium. The osmotic potential of the isolation medium was maintained optimally with about 6 percent sucrose, although a range of 2 percent to 12 percent is within the scope of this invention.

In an embodiment of the embryoid/callus organizing media, mineral salts concentration in IMC Culture Media "D" is (⅛x), the concentration which is used also in anther culture medium. The 6N1 salts major components have been modified to remove ammonium nitrogen. Osmotic potential in the culture medium is maintained with about 12 percent sucrose and about 400 mg/l proline. Silver nitrate (0.5 mg/l) was included in the medium to modify ethylene activity. The preculture media is further characterized by having a pH of about 5.7 to 6.0. Silver nitrate and vitamins do not appear to be crucial to this medium but do improve the efficiency of the response.

Whole anther cultures can also be used in the production of monocotyledonous plants from a plant culture system. There are some basic similarities of anther culture methods

and microspore culture methods with regard to the media used. A difference from isolated microspore cultures is that undisrupted anthers are cultured, so that a support, eg., a nylon mesh support, is not needed. The first step in developing the anther cultures is to incubate tassels at a cold temperature. A cold temperature is defined as less than about 25° C. More specifically, the incubation of the tassels is preferably performed at about 10° C. A range of 8 to 14° C. is also within the scope of the invention. The anthers are then dissected from the tassels, preferably after surface sterilization using forceps, and placed on solidified medium. An example of such a medium is designated by the inventors as 6N1-TGR-P4.

The anthers are then treated with environmental conditions that area combinations of stresses that are capable of diverting microspores from gametogenesis to embryogenesis. It is believed that the stress effect of sugar alcohols in the preculture medium, for example, mannitol, is produced by inducing starvation at the predefined temperature. In one embodiment, the incubation pretreatment is for about 14 days at 10° C. It was found that treating the anthers in addition with a carbon structure, an illustrative embodiment being a sugar alcohol, preferably, mannitol, produces dramatically higher anther culture response rates as measured by the number of eventually regenerated plants, than by treatment with either cold treatment or mannitol alone. These results are particularly surprising in light of teachings that cold is better than mannitol for these purposes, and that warmer temperatures interact with mannitol better.

To incubate the anthers, they are floated on a preculture medium which diverts the microspores from gametogenesis, preferably on a mannitol carbon structure,, more specifically, 0.3 M of mannitol plus 50 mg/l of ascorbic acid. 3 ml is about the total amount in a dish, for example, a tissue culture dish, more specifically, a 60 mm petri dish. Anthers are isolated from about 120 spikelets for one dish yields about 360 anthers.

Chromosome doubling agents can be used in the preculture media for anther cultures. Several techniques for doubling chromosome number (Jensen, 1974; Wan et al., 1989) have been described. Colchicine is one of the doubling agents. However, developmental abnormalities arising from in vitro cloning are further enhanced by colchicine treatments, and previous reports indicated that colchicine is toxic to microspores. The addition of colchicine in increasing concentrations during mannitol pretreatment prior to anther culture and microspore culture has achieved improved percentages.

An illustrative embodiment of the combination of a chromosome doubling agent and preculture medium is one which contains colchicine. In a specific embodiment, the colchicine level is preferably about 0.05 percent. The anthers remain in the mannitol preculture medium with the additives for about 10 days at 10° C. Anthers are then placed on maturation media, for example, that designated 6N1-TGR-P4, for 3 to 6 weeks to induce embryoids. If the plants are to be regenerated from the embryoids, shoot regeneration medium is employed, as in the isolated microspore procedure described in the previous sections. Other regeneration media can be used sequentially to complete regeneration of whole plants.

The anthers are then exposed to embryoid/callus promoting medium, for example, that designated 6N1-TGR-P4 to obtain callus or embryoids. The embryoids are recognized by identification visually of embryonic-like structures. At this stage, the embryoids are transferred serially to a series of regeneration media. In an illustrative embodiment, the shoot initiation medium comprises BAP (6-benzyl-aminopurine) and NAA (naphthalene acetic acid). Regeneration protocols for isolated microspore cultures and anther cultures are similar.

IX. OTHER CULTURES AND REGENERATION

The present invention contemplates a corn plant regenerated from a tissue culture of an inbred (e.g., 87DIA4) or hybrid plant (e.g., 4033843) of the present invention. As is well known in the art, tissue culture of corn can be used for the in vitro regeneration of a corn plant. By way of example, a process of tissue culturing and regeneration of corn is described in European Patent Application, publication 160, 390, the disclosure of which is incorporated by reference. Corn tissue culture procedures are also described in Green & Rhodes (1982) and Duncan et al., (1985). The study by Duncan et al. (1985) indicates that 97 percent of cultured plants produced calli capable of regenerating plants. Subsequent studies have shown that both inbreds and hybrids produced 91 percent regenerable calli that produced plants.

Other studies indicate that non-traditional tissues are capable of producing somatic embryogenesis and plant regeneration. See, e.g., Songstad et al. (1988); Rao et al. (1986); and Conger et al. (1987), the disclosures of which are incorporated herein by reference. Regenerable cultures may be initiated from immature embryos as described in PCT publication WO 95/06128, the disclosure of which is incorporated herein by reference.

Briefly, by way of example, to regenerate a plant of this invention, cells are selected following growth in culture. Where employed, cultured cells are preferably grown either on solid supports or in the form of liquid suspensions as set forth above. In either instance, nutrients are provided to the cells in the form of media, and environmental conditions are controlled. There are many types of tissue culture media comprising amino acids, salts, sugars, hormones and vitamins. Most of the media employed to regenerate inbred and hybrid plants have some similar components, the media differ in the composition and proportions of their ingredients depending on the particular application envisioned. For example, various cell types usually grow in more than one type of media, but exhibit different growth rates and different morphologies, depending on the growth media. In some media, cells survive but do not divide. Various types of media suitable for culture of plant cells have been previously described and discussed above.

An exemplary embodiment for culturing recipient corn cells in suspension cultures includes using embryogenic cells in Type II (Armstrong & Green, 1985; (Gordon-Kamm et al., 1990) callus, selecting for small (10 to 30 m) isodiametric, cytoplasmically dense cells, growing the cells in suspension cultures with hormone containing media, subculturing into a progression of media to facilitate development of shoots and roots, and finally, hardening the plant and readying it metabolically for growth in soil.

Meristematic cells (i.e., plant cells capable of continual cell division and characterized by an undifferentiated cytological appearance, normally found at growing points or tissues in plants such as root tips, stem apices, lateral buds, etc.) can be cultured.

Embryogenic calli are produced essentially as described in PCT Publication WO 95/06128. Specifically, inbred plants or plants from hybrids produced from crossing an inbred of the present invention with another inbred are grown to flowering in a greenhouse. Explants from at least one of the following F_1 tissues: the immature tassel tissue, intercalary meristems and leaf bases, apical meristems, immature ears and immature embryos are placed in an

initiation medium which contain MS salts, supplemented with thiamine, agar, and sucrose. Cultures are incubated in the dark at about 23° C. All culture manipulations and selections are performed with the aid of a dissecting microscope.

After about 5 to 7 days, cellular outgrowths are observed from the surface of the explants. After about 7 to 21 days, the outgrowths are subcultured by placing them into fresh medium of the same composition. Some of the intact immature embryo explants are placed on fresh medium. Several subcultures later (after about 2 to 3 months) enough material is present from explants for subdivision of these embryogenic calli into two or more pieces.

Callus pieces from different explants are not mixed. After further growth and subculture (about 6 months after embryogenic callus initiation), there are usually between 1 and 100 pieces derived ultimately from each selected explant. During this time of culture expansion, a characteristic embryogenic culture morphology develops as a result of careful selection at each subculture. Any organized structures resembling roots or root primordia are discarded. Material known from experience to lack the capacity for sustained growth is also discarded (translucent, watery, embryogenic structures). Structures with a firm consistency resembling at least in part the scutelum of the in vivo embryo are selected.

The callus is maintained on agar-solidified MS or N6-type media. A preferred hormone is 2,4-D. A second preferred hormone is dicamba. Visual selection of embryo-like structures is done to obtain subcultures. Transfer of material other than that displaying embryogenic morphology results in loss of the ability to recover whole plants from the callus.

Cell suspensions are prepared from the calli by selecting cell populations that appear homogeneous macroscopically. A portion of the friable, rapidly growing embryogenic calli is inoculated into MS or N6 Medium containing 2,4-D or dicamba. The calli in medium are incubated at about 27° C. on a gyrotary shaker in the dark or in the presence of low light. The resultant suspension culture is transferred about once every three to seven days, preferably every three to four days, by taking about 5 to 10 ml of the culture and introducing this inoculum into fresh medium of the composition listed above.

For regeneration, embryos which appear on the callus surface are selected and regenerated into whole plants by transferring the embryogenic structure, into a sequence of solidified media which include decreasing concentrations of 2,4-D or other auxins. Other hormones which can be used in culture media include dicamba, NAA, ABA, BAP, and 2-NCA. The reduction is relative to the concentration used in culture maintenance media. Plantlets are regenerated from these embryos by transfer to a hormone-free medium, subsequently transferred to soil, and grown to maturity.

Progeny are produced by taking pollen and selfing, backcrossing or sibling regenerated plants by methods well known to those skilled in the arts. Seeds are collected from the regenerated plants.

X. PROCESSES OF PREPARING CORN PLANTS AND THE CORN PLANTS PRODUCED BY SUCH CROSSES

The present invention also provides a process of preparing a novel corn plant and a corn plant produced by such a process. In accordance with such a process, a first parent corn plant is crossed with a second parent corn plant wherein at least one of the first and second corn plants is the inbred corn plant 87DIA4. In one embodiment, a corn plant prepared by such a process is a first generation F_1 hybrid corn plant prepared by a process wherein both the first and second parent corn plants are inbred corn plants.

Corn plants (*Zea mays L.*) can be crossed by either natural or mechanical techniques. Natural pollination occurs in corn when wind blows pollen from the tassels to the silks that protrude from the tops of the incipient ears. Mechanical pollination can be effected either by controlling the types of pollen that can blow onto the silks or by pollinating by hand.

In a preferred embodiment, crossing comprises the steps of:

(a) planting in pollinating proximity seeds of a first and a second parent corn plant, and preferably, seeds of a first inbred corn plant and a second, distinct inbred corn plant;

(b) cultivating or growing the seeds of the first and second parent corn plants into plants that bear flowers;

(c) emasculating flowers of either the first or second parent corn plant, i.e., treating the flowers so as to prevent pollen production, in order to produce an emasculated parent corn plant;

(d) allowing natural cross-pollination to occur between the first and second parent corn plant;

(e) harvesting seeds produced on the emasculated parent corn plant; and, where desired,

(f) growing the harvested seed into a corn plant, or preferably, a hybrid corn plant.

Parental plants are planted in pollinating proximity to each other by planting the parental plants in alternating rows, in blocks or in any other convenient planting pattern. Plants of both parental parents are cultivated and allowed to grow until the time of flowering. Advantageously, during this growth stage, plants are in general treated with fertilizer and, or other agricultural chemicals as considered appropriate by the grower.

At the time of flowering, in the event that plant 87DIA4, is employed as the male parent, the tassels of the other parental plant are removed from all plants employed as the female parental plant. The detasseling can be achieved manually but also can be done by machine, if desired.

The plants are then allowed to continue to grow and natural cross-pollination occurs as a result of the action of wind, which is normal in the pollination of grasses, including corn. As a result of the emasculation of the female parent plant, all the pollen from the male parent plant 87DIA4 is available for pollination because tassels, and thereby pollen bearing flowering parts, have been previously removed from all plants of the inbred plant being used as the female in the hybridization. Of course, during this hybridization procedure, the parental varieties are grown such that they are isolated from other corn fields to minimize or prevent my accidental contamination of pollen from foreign sources. These isolation techniques are well within the skill of those skilled in this art.

Both parental inbred plants of corn may be allowed to continue to grow until maturity or the male rows may be destroyed after flowering is complete. Only the ears from the female inbred parental plants are harvested to obtain seeds of a novel F_1 hybrid. The novel F_1 hybrid seed produced can then be planted in a subsequent growing season with the desirable characteristics in terms of F_1 hybrid corn plants providing improved grain yields and the other desirable characteristics disclosed herein, being achieved.

Alternatively, in another embodiment, both first and second parent corn plants can come from the same inbred corn plant, i.e., from the inbred designated 87DIA4. Thus, any corn plant produced using a process of the present invention and inbred corn plant 87DIA4, is contemplated by this invention. As used herein, crossing can mean selfing,

backcrossing, crossing to another or the same inbred, crossing to populations, and the like. All corn plants produced using the present inbred corn plant 87DIA4 as a parent are within the scope of this invention.

The utility of the inbred plant 87DIA4 also extends to crosses with other species. Commonly, suitable species will be of the family Graminaceae, and especially of the genera Zea, Tripsacum, Coix, Schlerachne, Polytoca, Chionachne, and Trilobachne, of the tribe Maydeae. Of these, Zea and Tripsacum, are most preferred. Potentially suitable for crosses with 87DIA4 can be the various varieties of grain sorghum, *Sorghum bicolor* (L.) Moench.

A. F_1 HYBRID CORN PLANT AND SEED PRODUCTION

Where the inbred corn plant 87DIA4 is crossed with another, different, corn inbred, a first generation (F_1) corn hybrid plant is produced. Both a F_1 hybrid corn plant and a seed of that F_1 hybrid corn plant are contemplated as aspects of the present invention.

Inbred 87DIA4 has been used to prepare an F_1 hybrid corn plant, designated 4033843.

The goal of a process of producing an F_1 hybrid is to manipulate the genetic complement of corn to generate new combinations of genes which interact to yield new, or improved traits (phenotypic characteristics). A process of producing an F_1 hybrid typically begins with the production of one or more inbred plants. Those plants are produced by repeated crossing of ancestrally related corn plants to try and concentrate certain genes within the inbred plants. The production of inbred 87DIA4 has been set forth hereinbefore.

Corn has a diploid phase which means two conditions of a gene (two alleles) occupy each locus (position on a chromosome). If the alleles are the same at a locus, there is said to be homozygosity. If they are different, there is said to be heterozygosity. In a completely inbred plant, all loci are homozygous. Because many loci when homozygous are deleterious to the plant, in particular leading to reduced vigor, less kernels, weak and/or poor growth, production of inbred plants is an unpredictable and arduous process. Under some conditions, heterozygous advantage at some loci effectively bars perpetuation of homozygosity.

Inbreeding requires coddling and sophisticated manipulation by human breeders. Even in the extremely unlikely event inbreeding rather than crossbreeding occurred in natural corn, achievement of complete inbreeding cannot be expected in nature due to well known deleterious effects of homozygosity and the large number of generations the plant would have to breed in isolation. The reason for the breeder to create inbred plants is to have a known reservoir of genes whose gametic transmission is at least somewhat predictable.

The development of inbred plants generally requires at least about 5 to 7 generations of selfing. Inbred plants are then cross-bred in an attempt to develop improved F_1 hybrids. Hybrids are then screened and evaluated in small scale field trials. Typically, about 10 to 15 phenotypic traits, selected for their potential commercial value, are measured. A selection index of the most commercially important traits is used to help evaluate hybrids. FACT, an acronym for Field Analysis Comparison Trial (strip trials), is an on-farm testing program employed by DEKALB Plant Genetics to perform the final evaluation of the commercial potential of a product.

During the next several years, a progressive elimination of hybrids occurs based on more detailed evaluation of their phenotype. Eventually, strip trials (FACT) are conducted to formally compare the experimental hybrids being developed with other hybrids, some of which were previously developed and generally are commercially successful. That is, comparisons of experimental hybrids are made to competitive hybrids to determine if there was any advantage to further commercial development of the experimental hybrids. Examples of such comparisons are presented in Section B, hereinbelow.

When the inbred parental plant 87DIA4 is crossed with another inbred plant to yield a hybrid (such as the hybrid 4033843), the original inbred can serve as either the maternal or paternal plant. For many crosses, the outcome is the same regardless of the assigned sex of the parental plants.

However, there is often one of the parental plants that is preferred as the maternal plant because of increased seed yield and production characteristics. Some plants produce tighter ear husks leading to more loss, for example due to rot. There can be delays in silk formation which deleteriously affect timing of the reproductive cycle for a pair of parental inbreds. Seed coat characteristics can be preferable in one plant. Pollen can be shed better by one plant. Other variables can also affect preferred sexual assignment of a particular cross.

B. F_1 HYBRID COMPARISONS

As mentioned in Section A, hybrids are progressively eliminated following detailed evaluations of their phenotype, including formal comparisons with other commercially successful hybrids. Strip trials are used to compare the phenotypes of hybrids grown in as many environments as possible. They are performed in many environments to assess overall performance of the new hybrids and to select optimum growing conditions. Because the corn is grown in close proximity, environmental factors that affect gene expression, such as moisture, temperature, sunlight and pests, are minimized. For a decision to be made that a hybrid is worth making commercially available, it is not necessary that the hybrid be better than all other hybrids. Rather, significant improvements must be shown in at least some, traits that would create improvements in some niches.

Examples of such comparative data are set forth hereinbelow in Table 6, which presents a comparison of performance data for the hybrid 4033843, a hybrid made with 87DIA4 as one parent, versus a selected hybrid of commercial value (DK442).

All the data in Table 6 represents results across years and locations for research and/or strip trials. The "NTEST" represents the number of paired observations in designated tests at locations around the United States.

TABLE 6

COMPARATIVE DATA FOR 4033843

HYBRID	NTEST	SI % C	YLD BU	MST PTS	STL %	RTL %	DRP %	FLSTD % M	SV RAT

TABLE 6-continued

COMPARATIVE DATA FOR 4033843

4033843	R 93	110.3	156.3	19.9	5.2	1.4	0.1	101.0	4.1
DK442		99.0	147.7	19.8	7.1	5.2	0.1	100.9	4.1
DEV		11.3**	8.6**	0.1	-1.9**	-3.8**	0.0	0.2	-0.1

HYBRID	NTEST	ELSTD % M	PHT INCH	EHT INCH	BAR %	SG RAT	TST LBS	FGDU	ESTR DAYS
4033843	R 93	104.4	89.8	40.9		5.0	54.2	1214.0	94.0
DK442		102.9	90.2	43.9		3.1	53.4	1251.0	93.9
DEV		1.5+	-0.4	-3.0**		1.8**	0.8**	-36.9**	0.1

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
LEGEND ABBREVIATIONS:
HYBD = Hybrid
TEST = Research/FACT
SI % C = Selection Index (percent of check)
YLD BU = Yield (bushels/acre)
MST PTS = Moisture
STL % = Stalk Lodging (percent)
RTL % = Root Lodging (percent)
DRP % = Dropped Ears (percent)
FLSTD % M = Final Stand (percent of test mean)
SV RAT = Seedling Vigor Rating
ELSTD % M = Early Stand (percent of test mean)
PHT INCH = Plant Height (inches)
EHT INCH = Ear Height (inches)
BAR % = Barren Plants (percent)
SG RAT = Staygreen Rating
TST LBS = Test Weight (pounds)
FGDU = GDUs to Shed
ESTR DAYS = Estimated Relative Maturity (days)

As can be seen in Table 6, the hybrid 4033843 has significantly higher yield with comparable moisture content when compared to a successful commercial hybrid. Significant differences are also shown in Table 6 for many other traits.

C. PHYSICAL DESCRIPTION OF F_1 HYBRIDS

The present invention also provides F_1 hybrid corn plants derived from the corn plant 87DIA4. Physical characteristics of exemplary hybrids are set forth in Table 7, which concerns 4033843, which has 87DIA4 as one inbred parent. An explanation of terms used in Table 7 can be found in the Definitions, set forth herein above.

TABLE 7

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996

	CHARACTERISTIC	VALUE
1.	STALK	
	Diameter (width) cm	2.6
	Anthocyanin	Absent
	Nodes with Brace Roots	1.5
	Brace Root Color	Red
	Internode Direction	Straight
	Internode Length cm.	16.0
2.	LEAF	
	Color	Med Green
	Length cm.	79.9
	Width cm.	10.7
	Sheath Anthocyanin	Absent
	Sheath Pubescence	Medium
	Marginal Waves	Medium
	Longitudinal Creases	Few

TABLE 7-continued

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996

	CHARACTERISTIC	VALUE
3.	TASSEL	
	Attitude	Compact
	Length cm.	48.1
	Spike Length cm.	27.4
	Peduncle Length cm.	12.1
	Branch Number	7.5
	Anther Color	Red
	Glume Color	Purple
	Glume Band	Absent
4.	EAR	
	Silk Color	Tan
	Number Per Stalk	1.1
	Position (attitude)	Upright
	Length cm.	20.7
	Shape	Semi-conical
	Diameter cm.	4.7
	Weight gm.	222.8
	Shank Length cm.	18.7
	Husk Bract	Short
	Husk Opening	Open
	Husk Color Fresh	Green
	Husk Color Dry	Buff
	Cob Diameter cm.	2.4
	Cob Color	Red
	Shelling Percent	88.6
5.	KERNEL	
	Row Number	15.4
	Number Per row	42.6
	Row Direction	Straight
	Type	Dent

TABLE 7-continued

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996	
CHARACTERISTIC	VALUE
Cap Color	Yellow
Side Color	Deep Yellow
Length (depth) mm.	12.0
Width mm.	7.9
Thickness	4.3
Weight of 1000K gm.	307.0
Endosperm Type	Normal
Endosperm Color	Yellow

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

XI. GENETIC COMPLEMENTS

In another aspect, the present invention provides a genetic complement of a plant of this invention. In one embodiment, therefore, the present invention contemplates an inbred genetic complement of the inbred corn plant designated 87DIA4. In another embodiment, the present invention contemplates a hybrid genetic complement formed by the combination of a haploid genetic complement from 87DIA4 and another haploid genetic complement. Means for determining a genetic complement are well-known in the art.

As used herein, the phrase "genetic complement" means an aggregate of nucleotide sequences, the expression of which sequences defines the phenotype of a corn plant or a cell or tissue of that plant. By way of example, a corn plant is genotyped to determine the array of the inherited markers it possesses. Markers are alleles at a single locus. They are preferably inherited in codominant fashion so that the presence of both alleles at a diploid locus is readily detectable, and they are free of environmental variation, i.e., their heritability is 1. This genotyping is preferably performed on at least one generation of the descendant plant for which the numerical value of the quantitative trait or traits of interest are also determined. The array of single locus genotypes is expressed as a profile of marker alleles, two at each locus. The marker allelic composition of each locus can be either homozygous or heterozygous. Homozygosity is a condition where both alleles at a locus are characterized by the same nucleotide sequence. Heterozygosity refers to different conditions of the gene at a locus. Markers that are used for purposes of this invention include restriction fragment length polymorphisms (RFLPs) and isozymes.

A plant genetic complement can be defined by genetic marker profiles that can be considered "fingerprints" of a genetic complement. For purposes of this invention, markers are preferably distributed evenly throughout the genome to increase the likelihood they will be near a quantitative trait loci (QTL) of interest (e.g., in tomatoes, Helentjaris et al., U.S. Pat. No. 5,385,835, Nienhuis et al., 1987). These profiles are partial projections of a sample of genes. One of the uses of markers in general is to exclude, or alternatively include, potential parents as contributing to offspring.

Phenotypic traits characteristic of the expression of a genetic complement of this invention are distinguishable by electrophoretic separation of DNA sequences cleaved by various restriction endonucleases. Those traits (genetic markers) are termed RFLPs (restriction fragment length polymorphisms).

Restriction fragment length polymorphisms (RFLPs) are genetic differences detectable by DNA fragment lengths, typically revealed by agarose gel electrophoresis, after restriction endonuclease digestion of DNA. There are large numbers of restriction endonucleases available, characterized by their nucleotide cleavage sites and their source, e.g., Eco RI. Variations in RFLPs result from nucleotide base pair differences which alter the cleavage sites of the restriction endonucleases, yielding different sized fragments.

Means for performing RFLP analyses are well known in the art. Restriction fragment length polymorphism analyses reported herein were conducted by Linkage Genetics. This service is available to the public on a contractual basis. Probes were prepared to the fragment sequences, these probes being complementary to the sequences thereby being capable of hybridizing to them under appropriate conditions well known to those skilled in the art. These probes were labeled with radioactive isotopes or fluorescent dyes for ease of detection. After the fragments were separated by size, they were identified by the probes. Hybridization with a unique cloned sequence permits the identification of a specific chromosomal region (locus). Because all alleles at a locus are detectable, RFLPs are codominant alleles, thereby satisfying a criteria for a genetic marker. They differ from some other types of markers, e.g., from isozymes, in that they reflect the primary DNA sequence, they are not products of transcription or translation. Furthermore, different RFLP genetic marker profiles result from different arrays of restriction endonucleases.

The RFLP genetic marker profile of each of the parental inbreds and exemplary resultant hybrids were determined. Because an inbred is essentially homozygous at all relevant loci, an inbred should, in almost all cases, have only one allele at each locus. In contrast, a diploid genetic marker profile of a hybrid should be the sum of those parents, e.g., if one inbred parent had the allele A at a particular locus, and the other inbred parent had B, the hybrid is AB by inference. Subsequent generations of progeny produced by selection and breeding are anticipated to be of genotype A, B, or AB for that locus position. When the F1 plant is used to produce an inbred, the locus should be either A or B for that position. Surprisingly, it has been observed that in certain instances, novel RFLP genotypes arise during the breeding process. For example, a genotype of C is observed at a particular locus position from the cross of parental inbreds with A and B at that locus. Such a novel RFLP genotype is observed for the 87DIA4, at least, for the RFLP markers M5213S and M8B2369S, as shown in Table 8. These novel RFLP markers further define the 87DIA4 inbred from the parental inbreds from which it was derived. An RFLP genetic marker profile of 87DIA4 is presented in Table 8.

TABLE 8

RFLP PROFILE OF 87DIA4				
PROBE/ENZYME	87DIA4	2FACC	3AZA1	AQA3
M0264H	D	G	G	D
M0306H	A	A	—	A
M0445E	C	B	B	C
M1120S	F	—	D	F
M1234H	D	D	E	E
M1236H	A	A	—	A
M1238H	A	A	F	K
M1401E	C	C	C	A
M1406H	A	—	B	B
M1447H	B	B	E	B
M1B725E	B	B	C	C
M2239H	A	A	C	C
M2297H	A	A	E	A

TABLE 8-continued

RFLP PROFILE OF 87DIA4

PROBE/ENZYME	87DIA4	2FACC	3AZA1	AOA3
M2298E	C	B	C	C
M2402H	E	E	E	E
M3212S	A	A	B	A
M3247E	D	B	D	D
M3257S	B	B	B	B
M3296H	D	A	D	D
M3432H	A	I	A	A
M3446S	C	B	C	C
M3457E	E	E	E	E
M4386H	B	B	A	A
M4396H	H	H	F	F
M4444H	B	B	A	A
M4UMC19H	A	A	A	A
M4UMC31S	D	A	B	D
M5213S	B	A	B	A
M5295E	C	D	C	C
M5408H	A	A	A	A
M5579S	B	B	B	B
M5UMC95H	A	A	B	B
M6223E	C	C	C	C
M6252H	D	—	D	E
M6280H	E	E	A	A
M6373E	A	E	A	A
M7263E	A	C	A	A
M7391H	C	C	A	A
M7392S	C	C	B	C
M7455H	A	A	C	C
M8110S	C	C	C	C
M8114E	B	B	E	E
M8268H	B	B	B	B
M8585H	A	A	A	A
M8B2369S	B	D	B	D
M8UMC48E	C	C	C	C
M9209E	C	C	A	A
M9266S	A	A	C	C
M9B713S	A	A	B	B
M2UMC34H	D	D	D	—
M6UMC85H	A	A	A	—
M9UMC94H	E	E	B	—
M3UM121X	C	C	C	—
M0UMC130	C	H	—	—

*Probes used to detect RFLPs are from Linkage Genetics, 1515 West 2200 South, Suite C, Salt Lake City, Utah 84119.

Another aspect of this invention is a plant genetic complement characterized by a genetic isozyme typing profile. Isozymes are forms of proteins that are distinguishable, for example, on starch gel electrophoresis, usually by charge and/or molecular weight. The techniques and nomenclature for isozyme analysis are described in, Stuber et al. (1988), which is incorporated by reference.

A standard set of loci can be used as a reference set. Comparative analysis of these loci is used to compare the purity of hybrid seeds, to assess the increased variability in hybrids compared to inbreds, and to determine the identity of seeds, plants, and plant parts. In this respect, an isozyme reference set can be used to develop genotypic "fingerprints."

Table 9 lists the identifying numbers of the alleles at isozyme loci types, and represents the exemplary genetic isozyme typing profile for 87DIA4.

TABLE 9

ISOZYME PROFILE OF 87DIA4

	ISOZYME ALLELE			
LOCUS	87DIA4	2FACC	3AZA1	AOA3
Acph1	2	2	4	4
Adh1	4	4	4	4
Cat3	9	9	9	9
Got1	4	4	4	4
Got2	4	4	4	4
Got3	4	4	4	4
Idh1	4	4	4	4
Idh2	6	6	6	6
Mdh1	6	6	6*	6
Mdh2	3.5	3.5	6	3
Mdh3	16	16	16	16
Mdh4	12	12	12	12
Mdh5	12	12	12	12
Pgm1	9	9	9	9
Pgm2	4	4	4	4
6Pgd1	3.8	3.8	3.8	3.8
6Pgd2	5	5	5	5
Phi1	4	4	4	5

*Allele is probably a 6, but null cannot be ruled out.

The present invention also contemplates a hybrid genetic complement formed by the combination of a haploid genetic complement of the corn plant 87DIA4 with a haploid genetic complement of a second corn plant. Means for combining a haploid genetic complement from the foregoing inbred with another haploid genetic complement can be any method hereinbefore for producing a hybrid plant from 87DIA4. It is also contemplated that a hybrid genetic complement can be prepared using in vitro regeneration of a tissue culture of a hybrid plant of this invention.

A hybrid genetic complement contained in the seed of a hybrid derived from 87DIA4 is a further aspect of this invention. Exemplary hybrid genetic complements are the genetic complements of the hybrid 4033843.

Table 10 shows the identifying numbers of the alleles for the hybrid 4033843, which are exemplary RFLP genetic marker profiles for hybrids derived from the inbred of the present invention. Table 10 concerns 4033843, which has 87DIA4 as one inbred parent.

TABLE 10

RFLP PROFILE FOR 4033843

Probe/Enzyme Combination	Allelic Pair
M0264H	DH
M0306H	AA
M0445E	BC
M1120S	EF
M1234H	AD
M1238H	AE
M1401E	AC
M1406H	AB
M1447H	BB
M1B725E	BB
M2239H	AD
M2297H	AC
M2298E	CC
M2402H	EE
M3212S	AC
M3257S	AB
M3296H	CD
M3432H	AA
M3446S	CF
M3457E	EE
M4386H	BD

TABLE 10-continued

RFLP PROFILE FOR 4033843

Probe/Enzyme Combination	Allelic Pair
M4396E	HH
M4444H	AB
M4UMC19H	AA
M4UMC31S	AD
M5213S	AB
M5408H	AA
M6223E	BC
M6252H	AD
M6280H	EG
M6373E	AE
M7263E	AA
M7391H	AC
M7392S	AC
M7455H	AB
M8110S	AC
M8114E	BB
M8268H	BL
M8585H	AB
M8B2369S	BB
M8UMC48E	CC
M9209E	AC
M9266S	AA
M9B713S	AA
M2UMC34H	DF
M9UMC94H	EE
M3UM121X	CD
M0UMC130	CC

*Probes used to detect RFLPs are from Linkage Genetics, 1515 West 2200 South, Suite C, Salt Lake City, Utah 84119.

The exemplary hybrid genetic complements of hybrid 4033843 may also be assessed by genetic isozyme typing profiles using a standard set of loci as a reference, set, using, e.g., the same, or a different, set of loci to those described above. Table 11 lists the identifying numbers of the alleles at isozyme loci types and presents the exemplary genetic isozyme typing profile for the hybrid 4033843, which is an exemplary hybrid derived from the inbred of the present invention. Table 11 concerns 4033843, which has 87D1A4 as one inbred parent.

TABLE 11

ISOZYME GENOTYPE FOR HYBRID 4033843

LOCUS	ISOZYME ALLELES
Acph1	2
Adh1	4
Cat3	9
Got1	4
Got2	4
Got3	4
Idh1	4
Idh2	6
Mdh1	6
Mdh2	3.5
Mdh3	16
Mdh4	12
Mdh5	12
Pgm1	9
Pgm2	4
6-Pgd1	3.8
6-Pgd2	5
Phi1	4

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of the foregoing illustrative embodiments, it will be apparent to those of skill in the art that variations, changes, modifications and alterations may be applied to the composition, methods, and in the steps or in the sequence of steps of the methods described herein, without departing from the true concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

Armstrong & Green, "Establishment and Maintenance of Friable Embryogenic Maize Callus and the Involvement of L-Proline," *Planta*, 164:207–214, 1985.

Conger et al., "Somatic Embryogenesis from Cultured Leaf Segments of *Zea Mays*," *Plant Cell Reports*, 6:345–347, 1987.

Duncan et al., "The Production of Callus Capable of Plant Regeneration from Immature Embryos of Numerous *Zea Mays* Genotypes," *Planta*, 165:322–332, 1985.

Fehr (ed.), *Principles of Cultivar Development*, Vol. 1: Theory and Technique, pp. 360–376, 1987.

Gaillard et al., "Optimization of Maize Microspore Isolation and Culture Condition for Reliable Plant Regeneration," *Plant Cell Reports*, 10(2):55, 1991.

Gordon-Kamm et al., "Transformation of Maize Cells and Regeneration of Fertile Transgenic Plants," *The Plant Cell*, 6:603–618, 1990.

Green & Rhodes, "Plant Regeneration in Tissue Cultures of Maize," *Maize for Biological Research*, Plant Molecular Biology Association, pp. 367–372, 1992.

Jensen, "Chromosome Doubling Techniques in Haploids," Haploids and Higher Plants—*Advances and Potentials, Proceedings of the First International Symposium*, University of Guelph, Jun. 10–14, 1974.

Nienhuis et al., "Restriction Fragment Length Polymorphism Analysis of Loci Associated with Insect Resistance in Tomato," *Crop Science*, 27:797–803, 1987.

Pace et al., "Anther Culture of Maize and the Visualization of Embryogenic Microspores by Fluorescent Microscopy," *Theoretical and Applied Genetics*, 73:83 63–869, 1987.

Poehlman & Sleper (eds), *Breeding Field Crops*, 4th Ed., pp. 172–175, 1995.

Rao et al., "Somatic Embryogenesis in Glume Callus Cultures," *Maize Genetics Cooperation Newsletter*, Vol. 60, 1986.

Songstad et al. "Effect of 1-Aminocyclopropate-1-Carboxylic Acid, Silver Nitrate, and Norbornadiene on Plant Regeneration from Maize Callus Cultures," *Plant Cell Reports*, 7:262–265, 1988.

Stuber et al., "Techniques and scoring procedures for starch gel electrophoresis of enzymes of maize *C. Zea mays, L.*," *Tech. Bull.*, N. Carolina Agric. Res. Serv., Vol. 286, 1988.

Wan et al., "Efficient Production of Doubled Haploid Plants Through Colchicine Treatment of Anther-

Derived Maize Callus," *Theoretical and Applied Genetics*, 77:889–892, 1989.

What is claimed is:

1. Inbred corn seed of the corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

2. The inbred corn seed of claim 1, further defined as an essentially homogeneous population of inbred corn seed designated 87DIA4.

3. The inbred corn seed of claim 1, further defined as essentially free from hybrid seed.

4. An inbred corn plant produced by growing the seed of an inbred corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

5. Pollen of the plant of claim 4.

6. An ovule of the plant of claim 4.

7. An essentially homogeneous population of corn plants produced by growing the seed of an inbred corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

8. A corn plant having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

9. The corn plant of claim 8, further comprising a cytoplasmic factor conferring male sterility.

10. A tissue culture of regenerable cells of inbred corn plaint 87DIA4, wherein the tissue regenerates plants having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

11. The tissue culture of claim 10, wherein the regenerable cells are embryos, meristematic cells, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks, stalks, or protoplasts or callus derived therefrom.

12. A corn plant regenerated from the tissue culture of claim 10, having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

13. An inbred corn plant cell of the corn plant of claim 4 having:

(a) an RFLP genetic marker profile in accordance with the profile shown in Table 8; or

(b) a genetic isozyme typing profile in accordance with the profile shown in Table 9.

14. The inbred corn plant cell of claim 13, having an RFLP genetic marker profile in accordance with the profile shown in Table 8.

15. The inbred corn plant cell of claim 13, having a genetic isozyme typing profile in accordance with the profile shown in Table 9.

16. The inbred corn plant cell of claim 13, having an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 8 and 9.

17. The inbred corn plant cell of claim 13, located within a corn plant or seed.

18. The inbred corn plant of claim 4 having:

(a) an RFLP genetic marker profile in accordance with the profile shown in Table 8; or

(b) a genetic isozyme typing profile in accordance with the profile shown in Table 9.

19. The inbred corn plant of claim 18, having an RFLP genetic marker profile in accordance with the profile shown in Table 8.

20. The inbred corn plant of claim 18, having a genetic isozyme typing profile in accordance with the profile shown in Table 9.

21. The inbred corn plant of claim 18, having an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 3 and 9.

22. A process of preparing corn seed, comprising crossing a first parent corn plant with a second parent corn plant, wherein said first or second corn plant is the inbred corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192, wherein seed is allowed to form.

23. The process of claim 22, further defined as a process of preparing hybrid corn seed, comprising crossing a first inbred corn plant with a second, distinct inbred corn plant, wherein said first or second corn plant is the inbred corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

24. The process of claim 23, wherein crossing comprises the steps of:

(a) planting in pollinating proximity seeds of said first and second inbred corn plants;

(b) cultivating the seeds of said first and second inbred corn plants into plants that bear flowers;

(c) emasculating the male flowers of said first or second inbred corn plant to produce an emasculated corn plant;

(d) allowing cross-pollination to occur between said first and second inbred corn plants; and

(e) harvesting seeds produced on said emasculated corn plant.

25. The process of claim 24, further comprising growing said harvested seed to produce a hybrid corn plant.

26. Hybrid corn seed produced by the process of claim 23.

27. A hybrid corn plant produced by the process of claim 25.

28. The hybrid corn plant of claim 27, wherein the plant is a first generation (F_1) hybrid corn plant.

29. The corn plant of claim 8, further comprising a single gene conversion.

30. The corn plant of claim 29, wherein the single gene was stably inserted into a corn genome by transformation.

31. The single gene conversion of the corn plant of claim 29, where the gene is a dominant allele.

32. The single gene conversion of the corn plant of claim 29, where the gene is a recessive allele.

33. The single gene conversion corn plant of claim 29, where the gene confers herbicide resistance.

34. The single gene conversion of the corn plant of claim 29, where the gene confers insect resistance.

35. The single gene conversion of the corn plant of claim 29, where the gene confers resistance to bacterial, fungal, or viral disease.

36. The single gene conversion of the corn plant of claim 29, wherein the gene confers male sterility.

37. The single gene conversion of the corn plant of claim 29, where the gene confers waxy starch.

38. The single gene conversion of the corn plant of claim 29, where the gene confers improved nutritional quality.

39. The single gene conversion of the corn plant of claim 29, where the gene confers enhanced yield stability.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145 Page 1 of 6

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 5, at lines 46-47, please delete "Ear-Fresh Husk The color of the husks 1 to 2 weeks after pollination scored as Color: green, red, or purple" and substitute therefor --Ear-Fresh Husk Color: The color of the husks 1 to 2 weeks after pollination scored as green, red, or purple--.

In col. 5, at lines 56-57, please delete "Ear-Number Per The average number of ears per plant Stalk:" and substitute therefor --Ear-Number Per Stalk: The average number of ears per plant--.

In col. 5, at lines 58-59, please delete "Ear-Shank The average number of internodes on the ear shank. Internodes:" and substitute therefor --Ear-Shank Internodes: The average number of internodes on the ear shank--.

In col. 6, at lines 51-53, please delete "Kernel-Aleurone The color of the aleurone scored as white, pink, tan, brown, Color: bronze, red, purple, pale purple, colorless, or variegated" and substitute therefor --Kernel-Aleurone Color: The color of the aleurone scored as white, pink, tan, brown, bronze, red, purple, pale purple, colorless, or variegated--.

In col. 6, at lines 57-58, please delete "Kernel-Endosperm The color of the endosperm scored as white, pale yellow, or Color: yellow" and substitute therefor --Kernel-Endosperm Color: The color of the endosperm scored as white, pale yellow, or yellow--.

In col. 6, at lines 59-60, please delete "Kernel-Endosperm The type of endosperm scored as normal, waxy, or opaque. Type:" and substitute therefor --Kernel-Endosperm Type: The type of endosperm scored as normal, waxy, or opaque--.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 6, at lines 65-66, please delete "Kernel-Number Per The average number of kernels in a single row. Row:" and substitute therefor --Kernel-Number Per Row: The average number of kernels in a single row--.

In col. 7, at lines 1-3, please delete "Kernel-Pericarp The color of the pericarp scored as colorless, red-white crown, tan, Color: bronze, brown, light red, cherry red, or variegated" and substitute therefor -- Kernel-Pericarp Color: The color of the pericarp scored as colorless, red-white crown, tan, bronze, brown, light red, cherry red, or variegated--.

In col. 7, at lines 4-6, please delete "Kernel-Row The direction of the kernel rows on the ear scored as straight, Direction: slightly curved, spiral, or indistinct (scattered)" and substitute therefor --Kernel-Row Direction: The direction of the kernel rows on the ear scored as straight, slightly curved, spiral, or indistinct (scattered)--.

In col. 7, at lines 30-33, please delete "Leaf-Longitudinal A rating of the number of longitudinal creases on the leaf surface 1 Creases: to 2 weeks after pollination. Creases are scored as absent, few, or many" and substitute therefor --Leaf-Longitudinal Creases: A rating of the number of longitudinal creases on the leaf surface 1 to 2 weeks after pollination. Creases are scored as absent, few, or many--.

In col. 7, at lines 34-36, please delete "Leaf-Marginal A rating of the waviness of the leaf margin 1 to 2 weeks after Waves: pollination. Rated as none, few, or many" and substitute therefor --Leaf-Marginal Waves: A rating of the waviness of the leaf margin 1 to 2 weeks after pollination. Rated as none, few, or many--.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 7, at lines 40-43, please delete "Leaf-Sheath A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks Anthocyanin: after pollination, scored as absent, basal-weak, basal-strong, weak or strong" and substitute therefor --Leaf-Sheath Anthocyanin: A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks after pollination, scored as absent, basal-weak, basal-strong, weak or strong--.

In col. 7, at lines 44-46, please delete "Leaf-Sheath A rating of the pubescence of the leaf sheath. Ratings are taken 1 Pubescence: to 2 weeks after pollination and scored as light, medium, or heavy" and substitute therefor --Leaf-Sheath Pubescence: A rating of the pubescence of the leaf sheath. Ratings are taken 1 to 2 weeks after pollination and scored as light, medium, or heavy--.

In col. 8, at lines 19-21, please delete "Stalk-Brace Root The color of the brace roots observed 1 to 2 weeks after pollination Color: as green, red, or purple" and substitute therefor --Stalk-Brace Root Color: The color of the brace roots observed 1 to 2 weeks after pollination as green, red, or purple--.

In col. 8, at lines 27-29, please delete "Stalk-Internode The direction of the stalk internode observed after pollination as Direction: straight or zigzag" and substitute therefor --Stalk-Internode Direction: The direction of the stalk internode observed after pollination as straight or zigzag--.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 8, at lines 30-31, please delete "Stalk-Internode The average length of the internode above the primary ear. Length:" and substitute therefor --Stalk Internode Length: The average length of the internode above the primary ear--.

In col. 8, at lines 35-36, please delete "Stalk-Internode With The average number of nodes having brace roots per plant. Brace Roots:" and substitute therefor --Stalk-Internode With Brace Roots: The average number of nodes having brace roots per plant--.

In col. 8, at lines 65-66, please delete "Tassel-Branch The average number of primary tassel branches. Number:" and substitute therefor --Tassel-Branch Number: The average number of primary tassel branches--.

In col. 9, at lines 7-9, please delete "Tassel-Peduncle The average length of the tassel peduncle, measured from the base of the flag leaf to the base Length: of the bottom tassel branch" and substitute therefor --Tassel-Peduncle Length: The average length of the tassel peduncle, measured from the base of the flag leaf to the base of the bottom tassel branch--.

In col. 13, at line 26, delete "3AZA1" and substitute therefor --AQA3--.

In col. 14, at line 52, delete "87DIA114" and substitute therefor --87DIA4--.

In col. 30, at line 4, , delete "DEKALB Plant Genetics" and substitute therefor --DEKALB Genetics Corporation--.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below.

At col. 17, in Table 5 delete all rows under "4. EAR" and ending with "Endosperm Color" and substitute therefor the following rows –

4. EAR				
Silk Color	Pink	Pink	—	Red
Number Per Stalk	1.0	1.0	1.0	1.4
Position (attitude)	Upright	—	—	Upright
Length cm.	14.6	16.0	14.6	15.6
Shape	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Diameter cm.	4.0	3.8	4.0	3.9
Weight gm.	104.9	100.6	103.2	107.6
Shank Length cm.	10.3	14.1	10.1	9.6
Husk Bract	Short	Short	Short	Short
Husk Cover cm.	6.4	2.5	4.4	3.7
Husk Color Fresh	Green	Green	Green	Green
Husk Color Dry	Buff	Buff	Buff	Buff
Cob Diameter cm.	2.3	2.4	2.3	2.1
Cob Color	Red	—	Red	Red
Shelling Percent	87.7	80.6	89.0	83.3

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

5. KERNEL				
Row Number	14.6	14.8	16.3	15.3
Number Per Row	32.1	27.1	29.3	29.7
Row Direction	Curved	Curved	Curved	Curved
Type	Dent	—	Dent	—
Cap Color	Yellow	Yellow	Yellow	Yellow
Side Color	Deep Yellow	—	Orange	
Length (depth) mm.	11.1	9.4	10.9	10.3
Width mm.	7.8	8.0	7.4	7.8
Thickness	3.9	5.2	4.4	4.2
Weight of 1000K gm.	269.0	252.4	233.0	247.8
Endosperm Type	Normal	Normal	Normal	Normal
Endosperm Color	Yellow	Yellow	Yellow	Yellow--.

Signed and Sealed this

Twenty-seventh Day of February, 2001

Attest:

Nicholas P. Godici

NICHOLAS P. GODICI

Attesting Officer

Acting Director of the United States Patent and Trademark Office

3/2/05, EAST Version: 2.0.1.4

EXHIBIT D

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

RE Status Check
Date(s) Docketed: Decision on Appeal
~~Rcvd; Response from Examiner~~
APR 0 4 2005 6/30/05
Client: DEKA:281US
Attorney(s): DLP, RFH
Initials:

Ex parte Thomas B. Carlson

Appeal No. 2004-2317[1]
Application No. 09/771,938

Heard: February 10, 2005[2]

MAILED

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U.S. PATENT AND TRADEMARK OFFICE
BOARD OF PATENT APPEALS
AND INTERFERENCES

Before SCHEINER, ADAMS and GREEN, Administrative Patent Judges.

ADAMS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on the appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 3, 6, 11, 14-20, 24, 25, and 27-31. The examiner has indicated that claims 1, 2, 5, 7-10, 12, 13 and 21-23 are allowable. Answer, page 2. Claims 4 and 26 are cancelled. Brief, page 2.

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[1] This appeal is substantially similar to Appeal No. 2004-1503, Application No. 09/606,808; Appeal No. 2004-1506, Application No. 09/771,938; Appeal No. 2004-1968, Application No. 10/00,0311; Appeal No. 2004-2343, Application No. 09/772,520; and Appeal No. 2005-0396, Application No. 10/077,589, which all share the same assignee, Monsanto Company, the parent of wholly-owned subsidiary DeKalb Genetics Corporation. Accordingly we have considered these appeals together.

[2] We note that examiner Ashwin Meta presented arguments at the oral hearing.

Claims 3, 6, 15, 16, 17, 27, 28, 30 and 31 are illustrative of the subject matter on appeal and are reproduced below. In addition, for convenience, we have reproduced allowable claims 2 and 5 below:

2. A population of seed of the corn variety I015036, wherein a sample of the seed of the corn variety I015036 was deposited under ATCC Accession No. PTA-3225.

3. The population of seed of claim 2, further defined as an essentially homogeneous population of seed.

5. A corn plant produced by growing a seed of the corn variety I015036, wherein a sample of the seed of the corn variety I015036 was deposited under ATCC Accession No. PTA-3225.

6. The corn plant of claim 5, having:
   (a) an SSR profile in accordance with the profile shown in Table 6; or
   (b) an isozyme typing profile in accordance with the profile shown in Table 7.

15. A corn plant capable of expressing all the physiological and morphological characteristics of the corn variety I015036, wherein a sample of the seed of the corn variety I015036 was deposited under ATCC Accession No. PTA-3225.

16. The corn plant of claim 15, further comprising a nuclear or cytoplasmic gene conferring male sterility.

17. A tissue culture of regenerable cells of a plant of corn variety I015036, wherein the tissue is capable of regenerating plants capable of expressing all the physiological and morphological characteristics of the corn variety I015036, wherein a sample of the seed of the corn variety I015036 was deposited under ATCC Accession No. PTA-3225.

27. The corn plant of claim 5, further defined as having a genome comprising a single locus conversion.

28. The corn plant of claim 27, wherein the single locus was stably inserted into a corn genome by transformation.

30. The corn plant of claim 27, wherein the locus confers a trait selected from the group consisting of herbicide tolerance; insect resistance; resistance to bacterial, fungal, nematode or viral disease; yield enhancement; waxy

starch; improved nutritional quality; enhanced yield stability; male sterility and restoration of male fertility.

31. A method of producing an inbred corn plant derived from the corn variety I015036, the method comprising the steps of:

    (a) preparing a progeny plant derived from the corn variety I015036 by crossing a plant of the corn variety I015036 with a second corn plant, wherein a sample of the seed of the corn variety I015036 was deposited under ATCC Accession No. PTA-3225;
    (b) crossing the progeny plant with itself or a second plant to produce a seed of a progeny plant of a subsequent generation;
    (c) growing a progeny plant of a subsequent generation from said seed and crossing the progeny plant of a subsequent generation with itself or a second plant;
    (d) repeating steps (b) and (c) for an addition[al] 3-10 generations to produce an inbred corn plant derived from the corn variety I015036.

The references relied upon by the examiner are:

Hunsperger et al. (Hunsperger)	5,523,520	Jun. 4, 1996

Eshed et al. (Eshed), "Less-Than-Additive Epistatic Interactions of Quantitative Trait Loci in Tomato," Genetics, Vol. 143, pp. 1807-17 (1996)

Kraft et al. (Kraft), "Linkage Disequilibrium and Fingerprinting in Sugar Beet," Theoretical and Applied Genetics, Vol. 101, pp. 323-36 (2000)

GROUNDS OF REJECTION

Claim 3 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "an essentially homogeneous population of seed."

Claim 14 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "[a]n essentially homogeneous population of corn plants produced by growing the seed of the corn variety I015036."

Claims 6 and 11 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "in accordance with."

Claims 15, and 17-20[3] stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "capable of expressing."

Claims 16 and 27-30[4] stand rejected under 35 U.S.C. § 112, second paragraph as failing to limit the scope of the claims from which they depend.

Claim 28 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of "the article 'a' in the recitation 'wherein the single locus was stably inserted into a corn genome.'"

Claim 30 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrases "yield enhancement," "improved nutritional quality," and "enhanced yield stability."

Claims 6, 11, 24, 25 and 27-31 stand rejected under the written description provision of 35 U.S.C. § 112, first paragraph.

Claims 27-30 stand rejected under the enablement provision of 35 U.S.C. § 112, first paragraph.

We reverse.

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[3] According to the examiner (Answer, page 13), since claim 18 depends from claims 17 it is included in this rejection. We also note, while the examiner lists (Answer, page 4) claim 19 as rejected under 35 U.S.C. § 112, second paragraph, the examiner fails to explain the basis of this rejection. Accordingly, we assume since claim 19 ultimately depends from claim 17, claim 19, like claim 18, was intended to be included in this rejection.

[4] According to the examiner (Answer, page 4), "[c]laims ... 27-30 ... stand rejected under 35 U.S.C. [§] 112, second paragrarph..." The examiner, however, provides no explanation as to why claim 29 is rejected. We can only assume that since claim 29, as well as claims 28 and 30, each depend from claim 27, they are rejected for the same reason as claim 27. Accordingly, we have included claims 28-30 with this ground of rejection.

BACKGROUND

The present "invention relates to inbred corn seed and plants of the variety designated I015036, and derivatives and tissue cultures thereof." Specification, page 1. According to appellant (specification, page 27), "[a] description of the physiological and morphological characteristics of corn plant I015036 is presented in Table 3" of the specification, pages 27-29. On this record the examiner has indicated that claims drawn to plants, plant parts, and seed of the corn variety designated I015036 are allowable. See e.g., claims 1, 2, 5, 7-10, 12 and 13, and Answer, page 2, wherein the examiner states "[c]laims 1, 2, 5, 7-10, 12 [and] 13 ... are allowed."

A second aspect of the present invention comprises hybrid plants and processes "for producing [first generation (F_1) hybrid[5]] corn seeds or plants, which ... generally comprise crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is a plant of the variety designated I015036." Specification, pages 7-9. On this record the examiner has indicated that claims drawn to a process of producing corn seed wherein the process comprises crossing a first parent corn plant with a second parent corn plant are allowable. See e.g., claims 21-23 and Answer, page 2, wherein the examiner states claims "21-23 are allowed."

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[5] According to the specification (page 21), a F_1 hybrid is "[t]he first generation progeny of the cross of two plants." During oral hearing, appellant confirmed that all claims drawn to hybrid plants or hybrid seeds (see e.g., claims 24 and 25) refer to F_1 hybrids.

A third aspect of the present invention comprises single locus converted plants of the corn variety I015036. Specification, page 6. As appellant explains (specification, page 23, emphasis added), single locus converted (conversion) plants are those plants

> which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. A single locus may comprise one gene, or in the case of transgenic plants, one or more transgenes integrated into the host genome at a single site (locus).

As appellant explains (specification, page 31):

> Many single locus traits have been identified that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques. Single locus traits may or may not be transgenic; examples of these traits include, but are not limited to, male sterility, waxy starch, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, male fertility, enhanced nutritional quality, industrial usage, yield stability, and yield enhancement. These genes are generally inherited through the nucleus, but may be inherited through the cytoplasm. Some known exceptions to this are genes for male sterility, some of which are inherited cytoplasmically, but still act as single locus traits.

A final aspect of the present invention is directed to a process of producing an inbred corn plant derived from a plant of the corn variety I015036. See e.g., claim 31. According to appellant's specification (bridging paragraph, pages 10-11),

> the present invention provides a method of producing an inbred corn plant derived from the corn variety I015036, the method comprising the steps of: (a) preparing a progeny plant derived from corn variety I015036, wherein said preparing comprises crossing a plant of the corn variety I015036 with a second corn plant, and

wherein a sample of the seed of corn variety I015036 has been deposited under ATCC Accession No. ... [PTA-3225]; (b) crossing the progeny plant with itself or a second plant to produce a seed of a progeny plant of a subsequent generation; (c) growing a progeny plant of a subsequent generation from said seed of a progeny plant of a subsequent generation and crossing the progeny plant of a subsequent generation with itself or a second plant; and (d) repeating steps (c) and (d) for an addition 3-10 generations to produce an inbred corn plant derived from the corn variety I015036. In the method, it may be desirable to select particular plants resulting from step (c) for continued crossing according to steps (b) and (c). By selecting plants having one or more desirable traits, an inbred corn plant derived from the corn variety I015036 is obtained which possesses some of the desirable traits of corn variety I015036 as well potentially other selected traits.

According to the examiner (Answer, page 36), "[t]he patentability of the method of claim 31 does not lie in the method steps, which require the simple acts of crossing corn plants, allowing progeny seed to be produced, and growing progeny plants from the seed...." Therefore, as we understand this aspect of the claimed invention (e.g., claim 31), the intent is not to claim a specific inbred corn plant resulting from the claimed process. See claim 31. Instead, as we understand it, claim 31 is drawn to a process wherein an inbred corn plant is derived from the corn variety I015036.

As appellant explains (specification, page 3),

The development of uniform corn plant hybrids requires the development of homozygous inbred plants, the crossing of these inbred plants, and the evaluation of the crosses. Pedigree breeding and recurrent selection are examples of breeding methods used to develop inbred plants from breeding populations. Those breeding methods combine the genetic backgrounds from two or more inbred plants or various other broad-based sources into breeding pools from which new inbred plants are developed by selfing and selection of desired phenotypes. The new inbreds are crossed with other inbred plants and the hybrids from these crosses are evaluated to determine which of those have commercial potential.

We emphasize, that while "new inbreds" having commercial potential may result from the method set forth in claim 31, the claim does not encompass any specific plant that is produced as a result of the method. Rather the claim encompasses only a method of producing an inbred corn plant that is "derived" from the corn variety I015036. The examiner has indicated that a claim drawn to a corn plant of the corn variety I015036 is allowable. See e.g., claim 5, and Answer, page 2, wherein the examiner states that claim 5 is allowed.

Against this backdrop, we now consider the rejections of record.

DISCUSSION

Definiteness:

Claims 3, 6, 11, 14-20 and 27-30 stand rejected under 35 U.S.C. § 112, second paragraph. For the following reasons we reverse.

Claim 3

Claim 3 depends from independent claim 2, and stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "an essentially homogeneous population of seed...." Answer, bridging paragraph, pages 4-5. According to the examiner (Answer, page 4), claim 2 is drawn to "'[a] population of seed of the corn variety I015036, wherein a sample of the seed of the corn variety I015036 was deposited under ATCC Accession No. PTA-3225." Thus, the examiner finds (Answer, page 5), the population of seed set forth in claim 2 "is a homogeneous population of seed of corn variety I015036."

Accordingly, the examiner finds (id.), "[t]he recitation, 'essentially homogeneous,' in claim 3 ... appear[s] to be superfluous."

However, as disclosed in appellant's specification (page 5),

> [e]ssentially homogeneous populations of inbred seed are those that consist essentially of the particular inbred seed, and are generally free from substantial numbers of other seed, so that the inbred seed forms between about 90% and about 100% of the total seed, and preferably, between about 95% and about 100% of the total seed.

Accordingly, we disagree with the examiner's assertion (Answer, page 6) that claim 3 is unclear simply because it may contain seed other than the seed of the corn variety I015036. We remind the examiner that claim language must be analyzed "not in a vacuum, but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary skill in the pertinent art." In re Moore, 439 F.2d 1232, 1235, 169 USPQ 236, 238 (CCPA 1971). Here, notwithstanding appellant's comments[6], it is our opinion that a person of ordinary skill in the art would recognize that an essentially homogeneous population of seed of the corn variety I015036 is a population of seed that is generally free from substantial numbers of other seed, e.g., wherein corn variety I015036 seed forms between about 90% and about 100% of the total seed in the population.[7]

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[6] According to appellant (Brief, page 7), an essentially homogeneous population of seed, is a population of seed that could be of non-uniform size and shape.

[7] Cf. the examiner's statement (Answer, page 6), "amending claim 3 to read '[a]n essentially homogeneous population of corn seeds consisting essentially of seed of claim 1', would obviate this rejection."

Accordingly, we reverse the rejection of claim 3 under 35 U.S.C. § 112, second paragraph.

Claim 14

Claim 14 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "[a]n essentially homogeneous population of corn plants produced by growing the seed of the corn variety I015036." Answer, page 6. According to the examiner (Answer, bridging paragraph, pages 6-7), "[t]he I015036 seed can only produce I015036 plants. ... [Therefore,] [t]he population can ... only consist of I015036 plants." Accordingly, the examiner finds it unclear "why the population is referred to as 'essentially homogeneous,' since such populations can comprise more than one variety of plant." Answer, page 7.

As appellant discloses (specification, page 6), "[t]he population of inbred corn seed of the invention can further be particularly defined as being essentially free from hybrid seed. The inbred seed population may be separately grown to provide an essentially homogeneous population of inbred corn plants designated I015036." As we understand the claim, growing the seed of claim 3, for example, would produce an essentially homogeneous population of corn plants of the corn variety I015036.[8]

[8] Cf. The examiner's statement (Answer, page 8), amending claim 14 "to read, '[a]n essentially homogeneous population of corn plants produced by growing a population of corn seed consisting essentially of the seed of corn plant I015036...' would obviate the rejection."

In addition, we direct the examiner's attention to Appeal No. 2005-0396, wherein a claim similar to claim 14 was presented for our review. In Appeal No. 2005-0396, the examiner of record indicated that claim 14, directed to "[a]n essentially homogeneous population of corn plants produced by growing the seed of the corn variety I180580...." was allowable. Accordingly, we find that the examiner has treated claim 14 in a manner that is inconsistent with the prosecution of claim 14 in 2005-0396. As we understand it, the only difference between claim 14 as it appears in Appeal No. 2005-0396 and the instant appeal is the variety of corn seed from which the plant is produced.

Accordingly we reverse the rejection of claim 14 under 35 U.S.C. § 112, second paragraph.

Claims 6 and 11

Claims 6 and 11 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "in accordance with." According to the examiner (Answer, page 9), it is unclear if a plant "that generally follows the trend of the profile of Table 6, but which differs at one or a few loci, [would] be considered in 'conformity' or 'in accordance' with the profile of Table 6."

On this record, we understand the phrase "in accordance with" as it is used in claims 6 and 11 to mean "the same"[9]. Stated differently, we understand the claims to read:

6. The corn plant of claim 5, having:
   (a) the same SSR profile as shown in Table 6; or

[9] During the February 10, 2005 oral hearing appellant's representative confirmed that the phrase "in accordance with" was intended to mean "the same."

(b) the same isozyme typing profile as shown in Table 7.

11. The plant part of claim 10, wherein said cell is further defined as having:
    (a) The same SSR profile as shown in Table 6; or
    (b) The same isozyme typing profile as shown in Table 7.

Accordingly we reverse the rejection of claims 6 and 11 under 35 U.S.C. § 112, second paragraph.

Claims 15 and 17-20

Claims 15, and 17-20 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "capable of expressing," or "capable of regenerating." According to the examiner (Answer, page 11), the claims do "not make clear if the plant actually expresses the traits, or when or under what conditions the traits are expressed." In this regard, the examiner finds (Answer, page 12),

> while the plant has the capacity to express the characteristics, for some reason it may not. Certain characteristics of a plant are expressed only at certain times of its life cycle, and are incapable of being expressed at other times. The colors of flower parts such as silks, or fruit parts such as husks, are examples. The promoters of many genes conferring traits require a transcription factor to become active. Is a plant that has such a gene, but not the transcription factor, considered "capable of expressing" that gene, and the trait associated with that gene, and is such a plant encompassed by the claims?

To address the examiner's concerns, we find it sufficient to state that if a plant has the capacity to express the claimed characteristics it meets the requirement of the claim regarding "capable of," notwithstanding that due to a particular phase of the life cycle the plant is not currently expressing a particular characteristic. Alternatively, if a plant is incapable of expressing the claimed

characteristics at any phase of the life cycle, because it lacks, for example, the "transcription factor" required for expression – such a plant would not meet the requirement of the claim regarding "capable of."

Here, we find the examiner's extremely technical criticism to be a departure from the legally correct standard of considering the claimed invention from the perspective of one possessing ordinary skill in the art. [10] In our opinion, a person of ordinary skill in the art would understand what is claimed. Amgen Inc. v. Chugai Pharmaceutical Co., Ltd., 927 F.2d 1200, 1217, 18 USPQ2d 1016, 1030 (Fed. Cir. 1991). We find the same to be true for the phrase "capable of" as set forth in claims 17-20.

Accordingly we reverse the rejection of claims 15, and 17-20 under 35 U.S.C. § 112, second paragraph.

Claims 16 and 27-30

Claims 16 and 27-30 stand rejected under 35 U.S.C. § 112, second paragraph as failing to limit the scope of the claims from which they depend. According to the examiner (Answer, page 9), since the plant set forth in claim 16 is male sterile it cannot express all the morphological and physiological characteristics of the male fertile corn variety I015036. Similarly, the examiner finds it unclear whether the plant set forth in claim 27 has all the characteristics of the plant set forth in claim 5, from which claim 27 depends. Id. In response,

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[10] Cf. Digital Equipment Corp. v. Diamond, 653 F.2d 701, 724, 210 USPQ 521, 546 (CA 1981).

appellant asserts (Brief, bridging paragraph, pages 8-9), claims 16 and 27 simply add a further limitation to the claims from which they depend. We agree.

For example, claim 16 reads on a corn plant capable of expressing all the physiological and morphological characteristics of the corn variety I015036, further comprising a nuclear or cytoplasmic gene conferring male sterility. In our opinion, the claims reasonably apprise those of skill in the art of their scope. Amgen. As set forth in Shatterproof Glass Corp. v. Libbey-Owens Ford Co., 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir. 1985), "[i]f the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more."

Accordingly we reverse the rejection of claims 16 and 27-30 under 35 U.S.C. § 112, second paragraph.

Claim 28

Claim 28 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of "the article 'a' in the recitation 'wherein the single locus was stably inserted into a corn genome.'" According to the examiner (Answer, page 13), "[t]he recitation does not make clear if the genome is that of I015036 or that of a different corn plant."

According to appellant's specification (page 23, emphasis removed), a "Single Locus Converted (Conversion) Plant" refers to

> [p]lants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in

> addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. A single locus may comprise one gene, or in the case of transgenic plants, one or more transgenes integrated into the host genome at a single site (locus).

Accordingly, we agree with appellant (Brief, page 10) "[t]he single locus referred to in claim 28 may or may not have been directly inserted into the genome of the claimed plant." As we understand the claim, and arguments of record, claim 28 presents two possibilities: (1) the single locus is directly inserted into the claimed plant and nothing further need be done; or (2) the single locus is directly inserted into a different plant, which is then used to transfer the single locus to the claimed plant through use of the plant breeding technique known as backcrossing.

In our opinion, the claim reasonably apprises those of skill in the art of its scope. Amgen. Accordingly, we reverse the rejection of claim 28 under 35 U.S.C. § 112, second paragraph.

Claim 30

Claim 30 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrases "yield enhancement," "improved nutritional quality," and "enhanced yield stability." According to the examiner the terms "yield enhancement," "improved nutritional quality," and "enhanced yield stability" are relative and have no definite meaning. Answer, page 14. The examiner is correct (Answer, page 14), when a word of degree is used appellant's specification must provide some standard for measuring that degree.

Seattle Box. Co. v. Industrial Crating & Packing, Inc., 731 F.2d 818, 826, 221 USPQ 568, 573-574 (Fed. Cir. 1984).

On this record, appellant asserts (Brief, page 11), it is "understood the enhancement of yield or yield stability and improved nutritional quality is relative to a plant lacking the single locus. The metes and bounds of the claim are thus fully understood by one of skill in the art and the use of the terms is not indefinite." On reflection, we agree with appellant. The fact that some claim language is not mathematically precise does not per se render the claim indefinite. Seattle Box. As set forth in Shatterproof Glass, "[i]f the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more." In our opinion, a person of ordinary skill in the art would have understood the enhancement of yield or yield stability and improved nutritional quality is relative to a plant lacking the single locus.

Accordingly we reverse the rejection of claim 30 under 35 U.S.C. § 112, second paragraph.

Written Description:

Claims 6, 11, 24, 25 and 27-31 stand rejected under 35 U.S.C. § 112, first paragraph, as the specification fails to adequately describe the claimed invention. For the following reasons, we reverse.

Claims 24 and 25[11]

Claims 24 and 25 both depend from claim 23. On this record, the examiner has indicated that claim 23 is allowable. Answer, page 2. The examiner finds (Answer, page 16), claims 24 and 25 are drawn to a hybrid plant or seed "produced by crossing inbred corn plant I015036 with any second, distinct inbred corn plant."

As we understand it, based on this construction of claims 24 and 25, the examiner is of the opinion that since the hybrids inherit only ½ of their diploid[12] set of chromosomes from the plant of corn variety I015036, a person of skill in the art would not have viewed the teachings of the specification as sufficient to demonstrate that appellant was in possession of the genus of hybrid seeds and plants encompassed by claims 24 and 25. According to the examiner (Answer, page 22), "[t]he fact that any hybrid plant will inherit half of its alleles from I015036 then does not provide sufficient description of the morphological and physiological characteristics expressed by the claimed hybrid plants."

There is no doubt that the expressed gene products of a hybrid plant, e.g., the morphological and physiological traits, of I015036 and a non-I015036 corn plant will depend on the combination of the genetic material inherited from both parents. See Answer, page 23. Nevertheless, we disagree with the examiner's

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[11] We recognize, as does the examiner (Answer, page 22) that appellant's reference to claims 22-26 (Brief, page 13) was intended to be a reference to claims 24 and 25.

[12] According to appellant's specification (page 21), diploid means "a cell or organism having two sets of chromosomes."

conclusion (id.) that "[t]he fact that any hybrid plant will inherit half of its alleles from I015036 then does not provide sufficient description of the morphological and physiological characteristics expressed by the claimed hybrid plants."

On these facts, we find it necessary to take a step back and consider what is claimed. The claims are drawn to a F_1 hybrid seed (claim 24) or plant (claim 25) resulting from a cross between a plant of corn variety I015036 and a non-I015036 corn variety. The claims do not require the hybrid to express any particular morphological or physiological characteristic. Nor do the claims require that a particular non-I015036 corn variety be used.[13] All that is required by the claims is that the hybrid has one parent that is a plant of corn variety I015036. Since the examiner has indicated that the seed and the plant of the corn variety I015036 are allowable (see claims 1 and 5), there can be no doubt that the specification provides an adequate written description of this corn variety. In addition, the examiner appears to recognize (Answer, page 25) that appellant's specification describes an exemplary hybrid wherein one parent was a plant of the corn variety I015036, see e.g., specification, pages 53-59. Accordingly, it is unclear to this merits panel what additional description is necessary.

As set forth in Reiffin v. Microsoft Corp., 214 F.3d 1342, 1345, 54 USPQ2d 1915, 1917 (Fed. Cir. 2000), the purpose of the written description

[13] According to appellant (Brief, page 15), "hundreds or even thousands of different inbred corn lines were well known to those of skill in the art prior to the filing [date] of the instant application, each of which could be crossed to make a hybrid plant with in the scope of the claims."

requirement is to "ensure that the scope of the right to exclude, as set forth in the claims does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification." Here the hybrid seed or plant has one parent that is a plant of the corn variety I015036. To that end, to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). For the foregoing reasons it is our opinion that appellant has provided an adequate written description of the subject matter set forth in claims 24 and 25.

We recognize the examiner's argument relating to SSR and isozyme markers (Answer, pages 25-29), as well as the examiner's arguments concerning a correlation between the hybrid's genome structure and the function of the hybrid plant (Answer, pages 23-25). However, for the foregoing reasons, we are not persuaded by these arguments.

Claims 6 and 11

Claims 6 and 11 depend ultimately upon claim 5. On this record, the examiner has indicated that claim 5 is allowable. Answer, page 2.

According to the examiner (Answer, page 8), while the specification provides the locus names and allele numbers of the SSR markers, the specification does not provide the actual nucleotide sequences that make up the markers. According to the examiner (Answer, page 18), "names of loci alone do not describe the structures of the markers themselves. Without a description of

the sequences of the markers, one cannot confirm their presence." In response, appellant points out (Brief, page 26), "the SSR markers were from Celera AgGen, Inc., which provides a commercial service for genotyping of maize varieties." In other words, a person of ordinary skill in the art could use the commercially available service provided by Celera AgGen, Inc. to determine whether a corn plant produced by growing a seed of the corn variety I015036 has an SSR profile which is the same as that shown in Table 6. Therefore, it is unclear to this panel why the examiner believes that such a disclosure fails to provide adequate written descriptive support for the claimed invention.[14] Accordingly, we are not persuaded by the examiner's argument.

Regarding the isozyme typing profile, the examiner finds (Answer, page 18), "Table 7 provides names of loci where isozyme markers reside, for three different corn plants, and a numerical value that represents the numbers of alleles at isozyme loci types. The nucleotide sequences that make up these loci are not described." In response, appellant points out (Brief, page 26), the isozyme "markers are well known and isozyme analysis in general [is] very well known having been used for decades." In this regard, we remind the examiner that the inquiry into whether the description requirement is met must be determined on a case-by-case basis and is a question of fact. In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). A description as filed is

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[14] We are not persuaded by the examiner's assertion (Answer, page 28) "that the [commercially available] service used to detect SSR markers is currently available is not a guarantee that it will remain so for the life of a patent issuing from the application." Cf. In re Metcalfe, 410 F.2d 1378, 1382, 161 USPQ 789, 792-3 (CCPA 1969).

presumed to be adequate; unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. See e.g., In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. Accordingly, it is the examiner who has the initial burden of establishing by a preponderance of evidence that a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. Wertheim, 541 F.2d at 263, 191 USPQ at 97. On this record, the examiner provides no evidence to support the assertion that simply because appellant has not provided the sequences that make up the loci for particular isozymes, appellant's specification does not adequately describe the claimed invention. Accordingly, we are not persuaded by the examiner's argument.

The examiner finds (Answer, page 37), claims 6 and 11 require that the claimed plant or plant cell exhibit either the claimed SSR profile or the isozyme profile. According to the examiner (id.), "[t]he genome of the cells of the I015036 seed deposited with the ATCC has both the SSR profile and the isozyme typing profile shown in Tables 5 and 6 for that plant. No plant is mentioned in the specification that has one genetic marker profile but not the other." The examiner's concern appears to be misplaced. To the extent that the examiner is concerned that the claim is open to read on a plant other than a corn plant produced by growing a seed of the corn variety I015036, we remind the

examiner that both claims 6 and 11 ultimately depend from claim 5[15], which is drawn to "[a] corn plant produced by growing a seed of the corn variety I015036...."

It appears that the examiner may have read claims 6 and 11 as drawn to a corn plant or plant cell having only one of the recited profiles. However, as we understand claims 6 and 11, determining whether the claimed corn plant (claim 6) or plant cell (claim 11) has one of the profiles does not mean that the plant, or plant cell would not also exhibit the other profile.

In addition, we direct the examiner's attention to claims 6 and 11 of Appeal No. 2005-0396. As we understand it, notwithstanding differences in the SSR and isozyme profiles, the disclosure in the specification as well as the language of the claims is substantially similar to that of the instant application. Nevertheless, the examiner in Appeal No. 2005-0396 apparently found that appellant's specification provided an adequate written description of the claimed invention as no rejection of claims 6 and 11 was made under the written description provision of 35 U.S.C. § 112, first paragraph in Appeal No. 2005-0396. Accordingly, we find that the examiner has treated claims 6 and 11 in a manner that is inconsistent with the prosecution of similar claims in related application 10/077,589, which is the subject matter of Appeal No. 2005-0396.

For the foregoing reasons, we are not persuaded by the examiner's arguments.

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[15] The examiner has indicated that claim 5 is allowable. Answer, page 2.

Claims 27-30

According to the examiner (Answer, page 18), "[c]laims 27-30 are drawn towards I015036 plants further comprising a single locus conversion, or wherein the single locus was stably inserted into a corn genome by transformation." The examiner finds, however, that "the specification does not describe identified or isolated single loci for all corn plant traits." Answer, page 19. More specifically, the examiner finds (id.), claims 27-30 "broadly encompass single loci that have not been discovered or isolated." To the extent that the examiner is asserting that appellant has not provided an enabling disclosure of single loci that have not been identified, we note that to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath.

Nevertheless, it may be that the examiner's concern (Answer, page 31), is that "single loci that alone govern 'yield enhancement' or 'enhanced yield stability' have not been discovered." In this regard, the examiner asserts (Answer, page 32), "the references cited in the specification do not describe isolated single genes or loci that confer yield enhancement or yield stability." Therefore, the examiner concludes (id.), "[a]ppellant cannot be in possession of plants further comprising single loci that have yet to be identified." The examiner, however, provides no evidence to support the assertion that a person of ordinary skill in the art would not recognize that single loci for yield enhancement or yield stability are known in the art. In this regard, we note that

appellant discloses (specification, page 31), "[m]any single locus traits have been identified ... examples of these traits include, but are not limited to, ... enhanced nutritional quality, industrial usage, yield stability, and yield enhancement." It appears that the examiner has overlooked appellant's assertion that single locus traits for yield stability and yield enhancement are well known in the art. To this end, we direct the examiner's attention to, for example, United States Patent No. 5,936,145 ('145)[16], issued August 10, 1999, which is prior to the filing date of the instant application. For clarity, we reproduce claims 8, 29 and 39 of the '145 patent below:

> 8. A corn plant having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.
>
> 29. The corn plant of claim 8, further comprising a single gene conversion.
>
> 39. The single gene conversion of the corn plant of claim 29, where the gene confers enhanced yield stability.

As we understand it, claim 39 of the '145 patent, is drawn to a corn plant which comprises a single gene conversion, wherein the gene confers enhanced yield stability. Thus, contrary to the examiner's assertion it appears, for example, that a single gene that confers enhanced yield stability was known in the art prior to the filing date of the instant application. We remind the examiner "a patent need not teach, and preferably omits, what is well known in the art." Hybritech

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[16] We note that the assignee of the '145 patent is DeKalb Genetics Corporation. The assignee of the present application is Monsanto Company, the parent of wholly-owned subsidiary DeKalb Genetics Corporation.

Incorporated v. Monoclonal Antibodies, Inc. 802 F.2d 1367, 1385, 231 USPQ 81, 94 (Fed. Cir. 1986).

We remind the examiner that the inquiry into whether the description requirement is met must be determined on a case-by-case basis and is a question of fact. Wertheim, 541 F.2d at 262, 191 USPQ at 96. A description as filed is presumed to be adequate; unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. See e.g., Marzocchi. The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. Accordingly, it is the examiner who has the initial burden of establishing by a preponderance of evidence that a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. Wertheim, 541 F.2d at 263, 191 USPQ at 97. On this record, the examiner provides no evidence to support the assertion that single loci that govern, for example, yield enhancement or enhanced yield stability are not described.

For the foregoing reasons, we are not persuaded by the examiner's arguments.

Claim 31

Claim 31 is drawn to a method of producing an inbred corn plant derived from the corn variety I015036. The claimed method begins by crossing a plant of the corn variety I015036 with any other corn plant. The method requires that the progeny corn plant be crossed either to itself, or with any other corn plant, and that the progeny of this cross be further crossed to itself, or with another corn

plant, and so on throughout several generations. As we understand it, claim 31, in its simplest form, is directed to a method of using a plant of the corn variety I015036 to produce an inbred corn plant.

Nevertheless, the examiner finds (Answer, page 20), "[a] review of the claim indicates that hybrid progeny of corn plant I015036 are required to perform further crosses, and that progeny of subsequent generations can be further outcrossed with different corn plants." Therefore, the examiner concludes (id.), "[t]he hybrid progeny of corn plant I015036, and progeny plants of subsequent generations, are essential to operate the claimed method." As we understand the examiner's argument, not only does appellant have to provide a written description of the starting corn plant (I015036), but appellant also must look into the future to determine every other potential corn plant that someone may wish to cross with the I015036 corn variety, and provide written descriptive support for not only every other corn plant that could be crossed with I015036, but also the resulting progeny of each cross.

As set forth in Reiffin, the purpose of the written description requirement is to "ensure that the scope of the right to exclude, as set forth in the claims does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification." Here the method of producing an inbred corn plant requires a plant of the corn variety I015036 be used as the starting material. To that end, to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added].

Vas-Cath. The examiner has indicated that a claim to a plant of the corn variety I015036 is allowable, see e.g., appellant's claim 5. Therefore, in our opinion, there can be no doubt that appellant was in possession of a plant of the corn variety I015036, in addition to a method of using that plant to cross with any other corn plant to produce an inbred corn plant as set forth in appellant's claim 31.

In our opinion, it matters not what the other corn plants are, or what the progeny of a cross between corn variety I015036 and some other corn plant represents. As the examiner explains (Answer, bridging paragraph, pages 20-21), patentability of the method of claim 31 "does not lie in the method steps, which require the simple acts of crossing corn plants, allowing progeny seed to be produced, and growing progeny plants from the seed...." In our opinion, patentability of the method of claim 31 does not lie in the various other or second corn plants either. In our opinion, patentiblity of the method of claim 31 lies in the use of the corn variety I015036. Accordingly, for the foregoing reasons, it is our opinion that appellant has "convey[ed] with reasonable clarity to those skilled in the art that, as of the filing date sought, [they were] in possession of the invention," Vas-Cath (emphasis omitted).

Summary

For the foregoing reasons, we reverse the rejection of claims 6, 11, 24, 25 and 27-31 under the written description provision of 35 U.S.C. § 112, first paragraph.

Enablement:

Claims 27-30 stand rejected under the enablement provision of 35 U.S.C. § 112, first paragraph. The examiner finds (Answer, page 39), claims 27-30 "are broadly drawn towards inbred corn plant I015036 further defined as having a genome comprising any single locus conversion, encoding any trait; or wherein the single locus was stably inserted into a corn genome by transformation." The examiner presents several lines of argument under this heading. We take each in turn.

I. Retaining all the morphological and physiological traits of I015036:

According to the examiner (Answer, page 38, emphasis added), "the specification does not teach any I015036 plants comprising a single locus conversion produced by backcrossing, wherein the resultant plant retains all of its morphological and physiological traits in addition to exhibiting the single trait conferred by the introduced single locus." With reference to Hunsperger, Kraft, and Eshed the examiner asserts (Answer, page 41), "[t]he rejection raises the issue of how linkage drag hampers the insertion of single genes alone into a plant by backcrossing, while recovering all of the original plant's genome."

We note, however, that claims 27-30 do not require that the single locus conversion plant retain all of the morphological and physiological traits of the parent plant in addition to exhibiting the single trait conferred by the introduction of the single loci. Nor do claims 27-30 require that the resultant plant retain all of the original plant's genome in addition to the single locus transferred into the

inbred via the backcrossing technique. As appellant explains (specification, bridging paragraph, pages 29-30, emphasis added),

> [t]he term single locus converted plant as used herein refers to those corn plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single locus transferred into the inbred via the backcrossing technique.

See also appellant's definition of single locus converted (conversion) plant at page 23 of the specification. We find nothing in the appellant's specification to indicate that the single locus converted plant retains all of the morphological and physiological traits, or all of the genome, of the parent plant in addition to the single locus transferred via the backcrossing technique. Accordingly, we disagree with the examiner's construction of claims 27-30 as "directed to exactly plant I015036, further comprising the single locus," which appears to disregard appellant's definition of a single locus converted plant. See Answer, page 43, emphasis added.

The examiner appears to appreciate (Answer, page 43) that appellant's specification provides an example of a converted plant. See e.g., specification, pages 35-36. However, for the foregoing reasons, we are not persuaded by the examiner's assertion (Answer, page 43) that the specification provides "no indication that all of the morphological and physiological traits of [this converted] ... corn plant were recovered, and that only one single locus was transferred from the donor plant." To the contrary, the examiner provides no evidence that the converted plant exemplified in appellant's specification did not retain

essentially all of the desired morphological and physiological characteristics of the inbred in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique.

Further, we recognize appellant's argument (Brief, page 27) that the examiner failed to establish a nexus between Hunsperger's discussion of petunias; Kraft's discussion of sugar beets; and Eshed's discussion of tomatoes, and the subject matter of the instant application - corn. Absent evidence to the contrary, we agree with appellant (id.), the examiner's opinion[17] that the references concerning petunias, sugar beets and tomatoes apply to corn is unsupported on this record. That the examiner has failed to identify (Answer, page 41) an example "in the prior art of plants in which linkage drag does not occur," does not mean that linkage drag is expected to occur in corn breeding, which according to appellant (Reply Brief, page 10) "is extremely advanced and well known in the art...." In this regard, we agree with appellant (Brief, page 28; Accord Reply Brief, page 11), the examiner has improperly placed the burden on appellant to demonstrate that the examiner's unsupported assertion is not true. We remind the examiner, as set forth in In re Wright, 999 F.2d 1557, 1561-62, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993):

> When rejecting a claim under the enablement requirement of section 112, the PTO bears an initial burden of setting forth a reasonable explanation as to why it believes that the scope of protection provided by that claim is not adequately enabled by the description of the invention provided in the specification of the application; this includes, of course, providing sufficient reasons for

[17] See Answer page 41, wherein the examiner asserts "[l]inkage drag appears to be a phenomenon that occurs in all plant types."

doubting any assertions in the specification as to the scope of enablement.

II. What plant is transformed in claim 28?

We recognize the examiner's assertion (Answer, page 39) that while claim 28 requires that a single locus be stably inserted into a corn genome by transformation, the claim does not indicate whether (1) the I015036 plant was transformed with the single locus, or (2) some other corn plant was transformed with the single locus and then introduced into I015036 by crossing. However, as appellant points out (Brief, page 12), claim 28 "specifies that the single locus was stably inserted into a corn genome. Loci that are stably inserted into a corn genome are also stably inherited. Thus the single locus need not have been inserted into the genome of corn variety I015036." Accordingly, the I015036 plant may be transformed with the single locus, or another plant may be transformed with the single locus and then introduced into I015036 by crossing.

It may be that the examiner is concerned that by transforming a non-I015036 plant with a single locus and then introducing this locus into I015036 by crossing would result in a plant that does not retain all of the morphological and physiological traits, or all of the genome, of the I015036 plant. For the foregoing reasons, however, this line of reasoning is not persuasive.

III. The single locus to be introduced:

The examiner finds (Answer, page 40), "the claims do not place any limit on the single locus to be introduced" into I015036 plants. The examiner recognizes, however, that "[t]he prior art shows that hundreds of nucleotide

sequences encoding products that confer various types of plant traits have been isolated at the time the instant invention was filed." Id. In addition, the examiner recognizes (id.), "[o]ne skilled in the art can transform any of these isolated nucleotide sequences known in the prior art into a corn plant cell, and regenerate a transgenic plant from the transformed cell."

Nevertheless, the examiner finds (id.), "[u]ndue experimentation would be required by one skilled in the art to isolate single loci that govern the traits encompassed by the claims." In this regard, the examiner asserts (Answer, page 44) that the claims broadly encompass corn plants comprising any type of single loci, including those that have not yet been identified or isolated. To the extent that the examiner is asserting that appellant has not provided an enabling disclosure of single loci that have not been identified, we note that enablement under 35 U.S.C. § 112, first paragraph is evaluated as of appellant's filing date. As set forth in Chiron Corp. v. Genentech Inc., 363 F.3d 1247, 1254, 70 USPQ2d 1321, 1325-26 (Fed. Cir. 2004), "a patent document cannot enable technology that arises after the date of application. The law does not expect an applicant to disclose knowledge invented or developed after the filing date. Such disclosure would be impossible. See In re Hogan, 559 F.2d 595, 605-06 [194 USPQ 527] (CCPA 1977)."

The examiner's comment, however, may be directed to his assertion (Answer, page 40) that "isolated loci whose products confer yield enhancement or enhanced yield stability (recited in claim 30), are not known in the prior art." However, as discussed, supra, it appears that contrary to the examiner's

assertion a single locus that confers the trait of, for example, yield enhancement was known in the art prior to the filing date of the instant invention. In addition, as discussed, supra, appellant's specification asserts that such traits were known in the art. See specification, page 31. Accordingly, as set forth in In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971), the burden is on

> the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure.

On this record, we find only the examiner's unsupported conclusions as to why the specification does not enable the claimed invention. We remind the examiner that nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples. Marzocchi, 439 F.2d at 223, 169 USPQ at 369. In the absence of an evidentiary basis to support the rejection, the examiner has not sustained his initial burden of establishing a prima facie case of non-enablement. In this regard, we note that the burden of proof does not shift to appellant until the examiner first meets his burden. Marzocchi, 439 F.2d at 223-224, 169 USPQ at 369-370.

We also recognize the examiner's assertion (Answer, pages 40-41) that claims 27-29 "encompass plants with single loci whose functions are unknown," or where the effects of expression of the single locus on the traits expressed by

I015036 are unknown. While this may be true, the examiner has not provided any evidence to suggest that it would require undue experimentation to obtain a single locus converted plant wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. See specification, page 23.

While it is not expressly stated in the text of the examiner's rejection, it may be that the examiner is concerned that the claims include inoperative embodiments. If so, the examiner is directed to Atlas Powder Co. v. E.I. DuPont De Nemours & Co., 750 F.2d 1569, 1576-77, 224 USPQ 409, 414 (Fed. Cir. 1984):

> Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. "It is not a function of the claims to specifically exclude ... possible inoperative substances...." In re Dinh-Nguyen, 492 F.2d 856, 859-59, 181 USPQ 46, 48 (CCPA 1974)(emphasis omitted). Accord, In re Geerdes, 491 F.2d 1260, 1265, 180 USPQ 789, 793 (CCPA 1974); In re Anderson, 471 F.2d 1237, 1242, 176 USPQ 331, 334-35 (CCPA 1971). Of course, if the number of inoperative combinations becomes significant, and in effect forces one of ordinary skill in the art to experiment unduly in order to practice the claimed invention, the claims might indeed be invalid. *See* e.g., In re Cook, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971).

On this record, the examiner provides no evidence that the number of inoperative embodiments is so large that a person of ordinary skill in the art would have to experiment unduly to practice the claimed invention. To the contrary, the examiner recognizes (Answer, page 40) that "[t]he prior art shows that hundreds of nucleotide sequences encoding products that confer various

types of plant traits have been isolated at the time the instant invention was filed"; and that "[o]ne skilled in the art can transform any of these isolated nucleotide sequences known in the prior art into a corn plant cell, and regenerate a transgenic plant from the transformed cell." Accordingly, we are not persuaded by the examiner's unsupported assertions.

For the foregoing reasons, we reverse the rejection of claims 27-30 under the enablement provision of 35 U.S.C. § 112, first paragraph.

SUMMARY

We reverse the rejection of claims 3, 6, 11, 14-20, and 27-30 under 35 U.S.C. § 112, second paragraph.

We reverse the rejection of claims 6, 11, 24, 25 and 27-31 under the written description provision of 35 U.S.C. § 112, first paragraph.

We reverse the rejection of claims 27-30 under the enablement provision of 35 U.S.C. § 112, first paragraph.

REVERSED

Toni R. Scheiner Administrative Patent Judge	
	BOARD OF PATENT
Donald E. Adams Administrative Patent Judge	APPEALS AND
	INTERFERENCES
Lora M. Green Administrative Patent Judge	

Robert E. Hanson
FULBRIGHT & JAWORSKI L.L.P.
A REGISTERED LIMITED LIABILITY PARTNERSHIP
600 CONGRESS AVENUE, SUITE 2400
AUSTIN, TX 78701

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	Examiner BPAI	Art Unit	Page of

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*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-5,936,145	08-1999	Bradbury	
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

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NON-PATENT DOCUMENTS

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Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

US005936145A

United States Patent [19]

Bradbury

[11] **Patent Number:** 5,936,145

[45] **Date of Patent:** Aug. 10, 1999

[54] **INBRED CORN PLANT 87DIA4 AND SEEDS THEREOF**

[75] Inventor: **Peter J. Bradbury**, Madison, Wis.

[73] Assignee: **DeKalb Genetics Corporation**, DeKalb, Ill.

[21] Appl. No.: **09/017,996**

[22] Filed: **Feb. 3, 1998**

Related U.S. Application Data

[60] Provisional application No. 60/037,305, Feb. 5, 1997.

[51] **Int. Cl.**[6] **A01H 5/00; A01H 4/00; A01H 1/00; C12N 5/04**

[52] **U.S. Cl.** **800/320.1; 800/298; 800/275; 800/271; 800/301; 800/302; 800/303; 435/412; 435/424; 435/430; 435/430.1**

[58] **Field of Search** 800/320.1, 298, 800/275, 271, 303, 274, 302; 435/172.3, 172.1, 412, 424, 430, 430.1

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Primary Examiner—Gary Benzion
Attorney, Agent, or Firm—Arnold, White & Durkee

[57] **ABSTRACT**

According to the invention, there is provided an inbred corn plant designated 87DIA4. This invention thus relates to the plants, seeds and tissue cultures of the inbred corn plant 87DIA4, and to methods for producing a corn plant produced by crossing the inbred plant 87DIA4 with itself or with another corn plant, such as another inbred. This invention further relates to corn seeds and plants produced by crossing the inbred plant 87DIA4 with another corn plant, such as another inbred, and to crosses with related species. This invention further relates to the inbred and hybrid genetic complements of the inbred corn plant 87DIA4, and also to the RFLP and genetic isozyme typing profiles of inbred corn plant 87DIA4.

39 Claims, No Drawings

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INBRED CORN PLANT 87DIA4 AND SEEDS THEREOF

The present application claims the priority of co-pending U.S. Provisional Patent Application Serial No. 60/037,305, filed Feb. 5, 1997, the entire disclosure of which is incorporated herein by reference without disclaimer.

BACKGROUND OF THE INVENTION

I. Technical Field of the Invention

The present invention relates to the field of corn breeding. In particular, the invention relates to the inbred corn seed and plant designated 87DIA4, and derivatives and tissue cultures of such inbred plant.

II. Description of the Background Art

The goal of field crop breeding is to combine various desirable traits in a single variety/hybrid. Such desirable traits include greater yield, better stalks, better roots, resistance to insecticides, herbicides, pests, and disease, tolerance to heat and drought, reduced time to crop maturity, better agronomic quality, and uniformity in germination times, stand establishment, growth rate, maturity, and fruit size.

Breeding techniques take advantage of a plant's method of pollination. There are two general methods of pollination: a plant self-pollinates if pollen from one flower is transferred to the same or another flower of the same plant. A plant cross-pollinates if pollen comes to it from a flower on a different plant.

Corn plants (*Zea mays L.*) can be bred by both self-pollination and cross-pollination. Both types of pollination involve the corn plant's flowers. Corn has separate male and female flowers on the same plant, located on the tassel and the ear, respectively. Natural pollination occurs in corn when wind blows pollen from the tassels to the silks that protrude from the tops of the ear shoot.

Plants that have been self-pollinated and selected for type over many generations become homozygous at almost all gene loci and produce a uniform population of true breeding progeny, a homozygous plant. A cross between two such homozygous plants produce a uniform population of hybrid plants that are heterozygous for many gene loci. Conversely, a cross of two plants each heterozygous at a number of gene loci produces a population of hybrid plants that differ genetically and are not uniform. The resulting non-uniformity makes performance unpredictable.

The development of uniform corn plant hybrids requires the development of homozygous inbred plants, the crossing of these inbred plants, and the evaluation of the crosses. Pedigree breeding and recurrent selection are examples of breeding methods used to develop inbred plants from breeding populations. Those breeding methods combine the genetic backgrounds from two or more inbred plants or various other broad-based sources into breeding pools from which new inbred plants are developed by selfing and selection of desired phenotypes. The new inbreds are crossed with other inbred plants and the hybrids from these crosses are evaluated to determine which of those have commercial potential.

The pedigree breeding method for single-gene traits involves crossing two genotypes. Each genotype can have one or more desirable characteristics lacking in the other; or, each genotype can complement the other. If the two original parental genotypes do not provide all of the desired characteristics, other genotypes can be included in the

breeding population. Superior plants that are the products of these crosses are selfed and selected in successive generations. Each succeeding generation becomes more homogeneous as a result of self-pollination and selection. Typically, this method of breeding involves five or more generations of selfing and selection: $S_1 \rightarrow S_2$; $S_2 \rightarrow S_3$; $S_3 \rightarrow S_4$; $S_4 \rightarrow S_5$, etc. After at least five generations, the inbred plant is considered genetically pure.

Backcrossing can also be used to improve an inbred plant. Backcrossing transfers a specific desirable trait from one inbred or other source to an inbred that lacks that trait. This can be accomplished for example by first crossing a superior inbred (A) (recurrent parent) to a donor inbred (non-recurrent parent), which carries the appropriate gene(s) for the trait in question. The progeny of this cross are then mated back to the superior recurrent parent (A) followed by selection in the resultant progeny for the desired trait to be transferred from the non-recurrent parent. After five or more backcross generations with selection for the desired trait, the progeny are heterozygous for loci controlling the characteristic being transferred, but are like the superior parent for most or almost all other genes. The last backcross generation would be selfed to give pure breeding progeny for the gene(s) being transferred.

A single cross hybrid corn variety is the cross of two inbred plants, each of which has a genotype which complements the genotype of the other. The hybrid progeny of the first generation is designated F_1. Preferred F_1 hybrids are more vigorous than their inbred parents. This hybrid vigor, or heterosis, is manifested in many polygenic traits, including markedly improved higher yields, better stalks, better roots, better uniformity and better insect and disease resistance. In the development of hybrids only the F_1 hybrid plants are sought. An F_1 single cross hybrid is produced when two inbred plants are crossed. A double cross hybrid is produced from four inbred plants crossed in pairs (A×B and C×D) and then the two F_1 hybrids are crossed again (A×B)×(C×D).

The development of a hybrid corn variety involves three steps: (1) the selection of plants from various germplasm pools; (2) the selfing of the selected plants for several generations to produce a series of inbred plants, which, although different from each other, each breed true and are highly uniform; and (3) crossing the selected inbred plants with unrelated inbred plants to produce the hybrid progeny (F_1). During the inbreeding process in corn, the vigor of the plants decreases. Vigor is restored when two unrelated inbred plants are crossed to produce the hybrid progeny (F_1). An important consequence of the homozygosity and homogeneity of the inbred plants is that the hybrid between any two inbreds is always the same. Once the inbreds that give a superior hybrid have been identified, hybrid seed can be reproduced indefinitely as long as the homogeneity of the inbred parents is maintained. Conversely, much of the hybrid vigor exhibited by F_1 hybrids is lost in the next generation (F_2). Consequently, seed from hybrid varieties is not used for planting stock. It is not generally beneficial for farmers to save seed of F_1 hybrids. Rather, farmers purchase F_1 hybrid seed for planting every year.

North American farmers plant over 70 million acres of corn at the present time and there are extensive national and international commercial corn breeding programs. A continuing goal of these corn breeding programs is to develop high-yielding corn hybrids that are based on stable inbred plants that maximize the amount of grain produced and minimize susceptibility to environmental stresses. To accomplish this goal, the corn breeder must select and develop superior inbred parental plants for producing hybrids.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a corn plant designated 87DIA4. Also provided are corn plants having all the physiological and morphological characteristics of corn plant 87DIA4.

The inbred corn plant of the invention may further comprise, or have, a cytoplasmic factor that is capable of conferring male sterility. Parts of the corn plant of the present invention are also provided, such as, e.g., pollen obtained from an inbred plant and an ovule of the inbred plant.

The invention also concerns seed of the corn plant 87DIA4, which has been deposited with the ATCC. The invention thus provides inbred corn seed designated 87DIA4, and having ATCC Accession No. 203192.

The inbred corn seed of the invention may be provided as an essentially homogeneous population of inbred corn seed designated 87DIA4.

Essentially homogeneous populations of inbred seed are those that consist essentially of the particular inbred seed, and are generally purified free from substantial numbers of other seed, so that the inbred seed forms between about 90% and about 100% of the total seed, and preferably, between about 95% and about 100% of the total seed. Most preferably, an essentially homogeneous population of inbred corn seed will contain between about 98.5%, 99%, 99.5% and about 100% of inbred seed, as measured by seed grow outs.

In any event, even if a population of inbred corn seed was found, for some reason, to contain about 50%, or even about 20% or 15% of inbred seed, this would still be distinguished from the small fraction of inbred seed that may be found within a population of hybrid seed, e.g., within a bag of hybrid seed. In such a bag of hybrid seed offered for sale, the Governmental regulations require that the hybrid seed be at least about 95% of the total seed. In the practice of the present invention, the hybrid seed generally forms at least about 97% of the total seed. In the most preferred practice of the invention, the female inbred seed that may be found within a bag of hybrid seed will be about 1% of the total seed, or less, and the male inbred seed that may be found within a bag of hybrid seed will be negligible, i.e., will be on the order of about a maximum of 1 per 100,000, and usually less than this value.

The population of inbred corn seed of the invention is further particularly defined as being essentially free from hybrid seed. The inbred seed population may be separately grown to provide an essentially homogeneous population of inbred corn plant designated 87DIA4.

In another aspect, the present invention provides for single gene converted plants of 87DIA4. The single transferred gene may preferably be a dominant or recessive allele. Preferably, the single transferred gene will confer such traits as male sterility, herbicide resistance, insect resistance, resistance for bacterial, fungal, or viral disease, male fertility, enhanced nutritional quality, and industrial usage. The single gene may be a naturally occurring maize gene or a transgene introduced through genetic engineering techniques.

In another aspect, the present invention provides a tissue culture of regenerable cells of inbred corn plant 87DIA4. The tissue culture will preferably be capable of regenerating plants having the physiological and morphological characteristics of the foregoing inbred corn plant, and of regenerating plants having substantially the same genotype as the foregoing inbred corn plant. Preferably, the regenerable cells in such tissue cultures will be embryos, protoplasts, meristematic cells, callus, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks or stalks. Still further, the present invention provides corn plants regenerated from the tissue cultures of the invention.

In yet another aspect, the present invention provides processes for preparing corn seed or plants, which processes generally comprise crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is the inbred corn plant designated 87DIA4. These processes may be further exemplified as processes for preparing hybrid corn seed or plants, wherein a first inbred corn plant is crossed with a second, distinct inbred corn plant to provide a hybrid that has, as one of its parents, the inbred corn plant 87DIA4.

In a preferred embodiment, crossing comprises planting, in pollinating proximity, seeds of the first and second parent corn plant, and preferably, seeds of a first inbred corn plant and a second, distinct inbred corn plant; cultivating or growing the seeds of said first and second parent corn plants into plants that bear flowers; emasculating the male flowers of the first or second parent corn plant, i.e., treating the flowers so as to prevent pollen production, in order to produce an emasculated parent corn plant; allowing natural cross-pollination to occur between the first and second parent corn plants; and harvesting the seeds from the emasculated parent corn plant. Where desired, the harvested seed is grown to produce a corn plant or hybrid corn plant.

The present invention also provides corn seed and plants produced by a process that comprises crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is the inbred corn plant designated 87DIA4. In one embodiment, corn plants produced by the process are first generation (F_1) hybrid corn plants produced by crossing an inbred in accordance with the invention with another, distinct inbred. The present invention further contemplates seed of an F_1 hybrid corn plant.

In certain exemplary embodiments, the invention provides an F_1 hybrid corn plant and seed thereof, which hybrid corn plant is designated 4033843, having 871DIA4 as one inbred parent.

In yet a further aspect, the invention provides an inbred genetic complement of the corn plant designated 87DIA4. The phrase "genetic complement" is used to refer to the aggregate of nucleotide sequences, the expression of which sequences defines the phenotype of, in the present case, a corn plant, or a cell or tissue of that plant. An inbred genetic complement thus represents the genetic make up of an inbred cell, tissue or plant, and a hybrid genetic complement represents the genetic make up of a hybrid cell, tissue or plant. The invention thus provides corn plant cells that have a genetic complement in accordance with the inbred corn plant cells disclosed herein, and plants, seeds and diploid plants containing such cells.

Plant genetic complements may be assessed by genetic marker profiles, and by the expression of phenotypic traits that are characteristic of the expression of the genetic complement, e.g., isozyme typing profiles. Thus, such corn plant cells may be defined as having an RFLP genetic marker profile in accordance with the profile shown in Table 8, or a genetic isozyme typing profile in accordance with the profile shown in Table 9, or having both an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 8 and 9.

In another aspect, the present invention provides hybrid genetic complements, as represented by corn plant cells, tissues, plants and seeds, formed by the combination of a haploid genetic complement of an inbred corn plant of the invention with a haploid genetic complement of a second corn plant, preferably, another, distinct inbred corn plant. In another aspect, the present invention provides a corn plant regenerated from a tissue culture that comprises a hybrid genetic complement of this invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. DEFINITIONS

Barren Plants: Plants that are barren, i.e., lack an ear with grain, or have an ear with only a few scattered kernels.

Cg: *Colletotrichum graminicola* rating. Rating times 10 is approximately equal to percent total plant infection.

CLN: Corn Lethal Necrosis (combination of Maize (Chlorotic Mottle Virus and Maize Dwarf Mosaic virus) rating: numerical ratings are based on a severity scale where 1=most resistant to 9=susceptible.

Cn: *Corynebacterium nebraskense* rating. Rating times 10 is approximately equal to percent total plant infection.

Cz: *Cercospora zeae-maydis* rating. Rating times 10 is approximately equal to percent total plant infection.

Dgg: *Diatraea grandiosella* girdling rating (values are percent plants girdled and stalk lodged).

Dropped Ears: Ears that have fallen from the plant to the ground.

Dsp: Diabrotica species root ratings (1=least affected to 9=severe pruning).

Ear-Attitude: The attitude or position of the ear at harvest scored as upright, horizontal, or pendant.

Ear-Cob Color: The color of the cob, scored as white, pink, red, or brown.

Ear-Cob Diameter: The average diameter of the cob measured at the midpoint.

Ear-Cob Strength: A measure of mechanical strength of the cobs to breakage, scored as strong or weak.

Ear-Diameter: The average diameter of the ear at its midpoint.

Ear-Dry Husk Color: The color of the husks at harvest scored as buff, red, or purple.

Ear-Fresh Husk The color of the husks 1 to 2 weeks after pollination scored as Color: green, red, or purple.

Ear-Husk Bract: The length of an average husk leaf scored as short, medium, or long.

Ear-Husk Cover: The average distance from the tip of the ear to the tip of the husks. Minimum value no less than zero.

Ear-Husk Opening: An evaluation of husk tightness at harvest scored as tight, intermediate, or open.

Ear-Length: The average length of the ear.

Ear-Number Per The average number of ears per plant. Stalk:

Ear-Shank The average number of internodes on the ear shank. Internodes:

Ear-Shank Length: The average length of the ear shank.

Ear-Shelling Percent: The average of the shelled grain weight divided by the sum of the shelled grain weight and cob weight for a single ear.

Ear-Silk Color: The color of the silk observed 2 to 3 days after silk emergence scored as green-yellow, yellow, pink, red, or purple.

Ear-Taper (Shape): The taper or shape of the ear scored as conical, semi-conical, or cylindrical.

Ear-Weight: The average weight of an ear.

Early Stand: The percent of plants that emerge from the ground as determined in the early spring.

ER: Ear rot rating (values approximate percent ear rotted).

Final Stand Count: The number of plants just prior to harvest.

GDUs to Shed: The number of growing degree units (GDUs) or heat units required for an inbred line or hybrid to have approximately 50 percent of the plants shedding pollen as measured from time of planting. Growing degree units are calculated by the Barger Method, where the heat units for a 24-hour period are calculated as GDUs=[Maximum daily temperature+Minimum daily temperature)/2]−50. The highest maximum daily temperature used is 86 degrees Fahrenheit and the lowest minimum temperature used is 50 degrees Fahrenheit. GDUs to shed is then determined by summing the individual daily values from planting date to the date of 50 percent pollen shed.

GDUs to Silk: The number of growing degree units for an inbred line or hybrid to have approximately 50 percent of the plants with silk emergence as measured from time of planting. Growing degree units are calculated by the same methodology as indicated in the GDUs to shed definition.

Hc2: *Helminthosporium carbonum* race 2 rating. Rating times 10 is approximately equal to percent total plant infection.

Hc3: *Helminthosporium carbonum* race 3 rating. Rating times 10 is approximately equal to percent total plant infection.

Hm: *Helminthosporium maydis* race 0 rating. Rating times 10 is approximately equal to percent total plant infection.

Ht1: *Helminthosporium turcicum* race 1 rating. Rating times 10 is approximately equal to percent total plant infection.

Ht2: *Helminthosporium turcicum* race 2 rating. Rating times 10 is approximately equal to percent total plant infection.

HtG: +=Presence of Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection. −=Absence of a Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection. +/−=Segregation of a Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection.

Kernel-Aleurone The color of the aleurone scored as white, pink, tan, brown, Color: bronze, red, purple, pale purple, colorless, or variegated.

Kernel-Cap Color: The color of the kernel cap observed at dry stage, scored as white, lemon-yellow, yellow or orange.

Kernel-Endosperm The color of the endosperm scored as white, pale yellow, or Color: yellow.

Kernel-Endosperm The type of endosperm scored as normal, waxy, or opaque. Type:

Kernel-Grade: The percent of kernels that are classified as rounds.

Kernel-Length: The average distance from the cap of the kernel to the pedicel.

Kernel-Number Per The average number of kernels in a single row. Row:

Kernel-Pericarp The color of the pericarp scored as colorless, red-white crown, tan, Color: bronze, brown, light red, cherry red, or variegated.

Kernel-Row The direction of the kernel rows on the ear scored as straight, Direction: slightly curved, spiral, or indistinct (scattered).

Kernel-Row Number: The average number of rows of kernels on a single ear.

Kernel-Side Color: The color of the kernel side observed at the dry stage, scored as white, pale yellow, yellow, orange, red, or brown.

Kernel-Thickness: The distance across the narrow side of the kernel.

Kernel-Type: The type of kernel scored as dent, flint, or intermediate.

Kernel-Weight: The average weight of a predetermined number of kernels.

Kernel-Width: The distance across the flat side of the kernel.

Kz: *Kabatiella zeae* rating. Rating times 10 is approximately equal to percent total plant infection.

Leaf-Angle: Angle of the upper leaves to the stalk scored as upright (0 to 30 degrees), intermediate (30 to 60 degrees), or lax (60 to 90 degrees).

Leaf-Color: The color of the leaves 1 to 2 weeks after pollination scored as light green, medium green, dark green, or very dark green.

Leaf-Length: The average length of the primary ear leaf.

Leaf-Longitudinal A rating of the number of longitudinal creases on the leaf surface 1 Creases: to 2 weeks after pollination. Creases are scored as absent, few, or many.

Leaf-Marginal A rating of the waviness of the leaf margin 1 to 2 weeks after Waves: pollination. Rated as none, few, or many.

Leaf-Number: The average number of leaves of a mature plant. Counting begins with the cotyledonary leaf and ends with the flag leaf.

Leaf-Sheath A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks Anthocyanin: after pollination, scored as absent, basal-weak, basal-strong, weak or strong.

Leaf-Sheath A rating of the pubescence of the leaf sheath. Ratings are taken 1 Pubescence: to 2 weeks after pollination and scored as light, medium, or heavy.

Leaf-Width: The average width of the primary ear leaf measured at its widest point.

LSS: Late season standability (values times 10 approximate percent plants lodged in disease evaluation plots).

Moisture: The moisture of the grain at harvest.

On1: *Ostrinia nubilalis* 1st brood rating (1=resistant to 9=susceptible).

On2: *Ostrinia nubilalis* 2nd brood rating (1=resistant to 9=susceptible).

Relative Maturity: A maturity rating based on regression analysis. The regression analysis is developed by utilizing check hybrids and their previously established day rating versus actual harvest moistures. Harvest moisture on the hybrid in question is determined and that moisture value is inserted into the regression equation to yield a relative maturity.

Root Lodging: Root lodging is the percentage of plants that root lodge. A plant is counted as root lodged if a portion of the plant leans from the vertical axis by approximately 30 degrees or more.

Seedling Color: Color of leaves at the 6 to 8 leaf stage.

Seedling Height: Plant height at the 6 to 8 leaf stage.

Seedling Vigor: A visual rating of the amount of vegetative growth on a 1 to 9 scale, where 1 equals best. The score is taken when the average entry in a trial is at the fifth leaf stage.

Selection Index: The selection index gives a single measure of hybrid's worth based on information from multiple traits. One of the traits that is almost always included is yield. Traits may be weighted according to the level of importance assigned to them.

Sr: *Sphacelotheca reiliana* rating is actual percent infection.

Stalk-Anthocyanin: A rating of the amount of anthocyanin pigmentation in the stalk. The stalk is rated 1 to 2 weeks after pollination as absent, basal-weak, basal-strong, weak, or strong.

Stalk-Brace Root The color of the brace roots observed 1 to 2 weeks after pollination Color: as green, red, or purple.

Stalk-Diameter: The average diameter of the lowest visible internode of the stalk.

Stalk-Ear Height: The average height of the ear measured from the ground to the point of attachment of the ear shank of the top developed ear to the stalk.

Stalk-Internode The direction of the stalk internode observed after pollination as Direction: straight or zigzag.

Stalk-Internode The average length of the internode above the primary ear. Length:

Stalk Lodging: The percentage of plants that did stalk lodge. Plants are counted as stalk lodged if the plant is broken over or off below the ear.

Stalk-Nodes With The average number of nodes having brace roots per plant. Brace Roots:

Stalk-Plant Height: The average height of the plant as measured from the soil to the tip of the tassel.

Stalk-Tillers: The percent of plants that have tillers. A tiller is defined as a secondary shoot that has developed as a tassel capable of shedding pollen.

Staygreen: Staygreen is a measure of general plant health near the time of black layer formation (physiological maturity). It is usually recorded at the time the ear husks of most entries within a trial have turned a mature color. Scoring is on a 1 to 9 basis where 1 equals best.

STR: Stalk rot rating (values represent severity rating of 1=25 percent of inoculated internode rotted to 9=entire stalk rotted and collapsed).

SVC: Southeastern Virus Complex combination of Maize Chlorotic Dwarf Virus and Maize Dwarf Mosaic Virus) rating; numerical ratings are based on a severity scale where 1=most resistant to 9=susceptible (1988 reactions are largely Maize Dwarf Mosaic Virus reactions).

Tassel-Anther Color: The color of the anthers at 50 percent pollen shed scored as green-yellow, yellow, pink, red, or purple.

Tassel-Attitude: The attitude of the tassel after pollination scored as open or compact.

Tassel-Branch Angle: The angle of an average tassel branch to the main stem of the tassel scored as upright (less than 30 degrees), intermediate (30 to 45 degrees), or lax (greater than 45 degrees).

Tassel-Branch The average number of primary tassel branches. Number:

Tassel-Glume Band: The closed anthocyanin band at the base of the glume scored as present or absent.

Tassel-Glume Color: The color of the glumes at 50 percent shed scored as green, red, or purple.

Tassel-Length: The length of the tassel measured from the base of the bottom tassel branch to the tassel tip.

Tassel-Peduncle The average length of the tassel peduncle, measured from the base Length: of the flag leaf to the base of the bottom tassel branch.

Tassel-Pollen Shed: A visual rating of pollen shed determined by tapping the tassel and observing the pollen flow of approximately five plants per entry. Rated on a 1 to 9 scale where 9=sterile, 1=most pollen.

Tassel-Spike Length: The length of the spike measured from the base of the top tassel branch to the tassel tip.

Test Weight: The measure of the weight of the grain in pounds for a given volume (bushel) adjusted to 15.5 percent moisture.

Yield: Yield of grain at harvest adjusted to 15.5 percents moisture.

II. OTHER DEFINITIONS

Allele is any of one or more alternative forms of a gene, all of which alleles relate to one trait or characteristic. In a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes.

Backcrossing is a process in which a breeder repeatedly crosses hybrid progeny back to one of the parents, for example, a first generation hybrid (F_1) with one of the parental genotypes of the F1 hybrid.

Chromatography is a technique wherein a mixture of dissolved substances are bound to a solid support followed by passing a column of fluid across the solid support and varying the composition of the fluid. The components of the mixture are separated by selective elution.

Crossing refers to the mating of two parent plants.

Cross-pollination refers to fertilization by the union of two gametes from different plants.

Diploid refers to a cell or organism having two sets of chromosomes.

Electrophoresis is a process by which particles suspended in a fluid are moved under the action of an electrical field, and thereby separated according to their charge and molecular weight. This method of separation is well known to those skilled in the art and is typically applied to separating various forms of enzymes and of DNA fragments produced by restriction endonucleases.

Emasculate refers to the removal of plant male sex organs.

Enzymes are organic catalysts that can exist in various forms called isozymes.

F_1 Hybrid refers to the first generation progeny of the cross of two plants.

Genetic Complement refers to an aggregate of nucleotide sequences, the expression of which sequences defines the phenotype in corn plants, or components of plants including cells or tissue.

Genotype refers to the genetic constitution of a cell or organism.

Haploid refers to a cell or organism having one set of the two sets of chromosomes in a diploid.

Isozymes are one of a number of enzymes which catalyze the same reaction(s) but differ from each other, e.g., in primary structure and/or electrophoretic mobility. The differences between isozymes are under single gene, codominant control. Consequently, electrophoretic separation to produce band patterns can be equated to different alleles at the, DNA level. Structural differences that do not alter charge cannot be detected by this method.

Isozyme typing profile refers to a profile of band patterns of isozymes separated by electrophoresis that can be equated to different alleles at the DNA level.

Linkage refers to a phenomenon wherein alleles on the same chromosome tend to segregate together more often than expected by chance if their transmission was independent.

Marker is a readily detectable phenotype, preferably inherited in codominant fashion (both alleles at a locus in a diploid heterozygote are readily detectable), with no environmental variance component, i.e., heritability of 1.

87DIA4 refers to the corn plant from which seeds having ATCC Accession No. 203192 were obtained, as well as plants grown from those seeds.

Phenotype refers to the detectable characteristics of a cell or organism, which characteristics are the manifestation of gene expression.

Quantitative Trait Loci (QTL) refer to genetic loci that control to some degree numerically representable traits that are usually continuously distributed.

Regeneration refers to the development of a plant from tissue culture.

RFLP genetic marker profile refers to a profile of band patterns of DNA fragment lengths typically separated by agarose gel electrophoresis, after restriction endonuclease digestion of DNA.

Self-pollination refers to the transfer of pollen from the anther to the stigma of the same plant.

Single Gene Converted (Conversion) Plant refers to plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single gene transferred into the inbred via the backcrossing technique.

Tissue Culture refers to a composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

III. INBRED CORN PLANT 87DIA4

In accordance with one aspect of the present invention, there is provided a novel inbred corn plant, designated 87DIA4. Inbred corn plant 87DIA4 is a yellow, dent corn inbred that can be compared to inbred corn plants 2FACC, 3AZA1, and AQA3, all of which are proprietary inbreds of DEKALB Genetics Corporation. 87DIA4 differs significantly (at the 1%, 5%, or 10% level) from these inbred lines in several aspects (Table 1, Table 2, and Table 3).

TABLE 1

COMPARISON OF 87DIA4 WITH 2FACC

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
2FACC	0.4	0.6	29.5	62.0	23.9	67.1	1.3	1482.6	1481.5	5.8	77.4
DIFF	-0.1	-0.5	-5.2	0.4	-6.1	-10.1	-1.1	-119.5	-124.4	2.4	-12.3
# LOC	15	13	8	15	14	8	14	8	8	13	13
P VALUE	0.88	0.65	0.00**	0.84	0.00**	0.00**	0.40	0.00**	0.00**	0.36	0.01*

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

TABLE 2

COMPARISON OF 87DIA4 WITH 3AZA1

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
3AZA1	1.1	1.6	23.6	62.0	15.9	58.4	0.1	1322.6	1321.2	8.4	41.1
DIFF	-0.8	-1.5	0.7	0.4	1.9	-1.4	0.1	40.5	35.9	-0.2	24.0
# LOC	15	13	8	15	14	8	14	8	8	13	13
P VALUE	0.41	0.19	0.66	0.84	0.13	0.48	0.94	0.00**	0.03*	0.94	0.00**

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

TABLE 3

COMPARISON OF 87DIA4 WITH AQA3

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
AQA3	0.4	0.5	25.9	62.7	14.4	58.1	1.0	1356.2	1348.6	15.2	35.7
DIFF	-0.1	-0.4	-1.6	-0.3	3.3	-1.1	-0.8	6.9	8.5	-7.0	29.4

TABLE 3-continued

COMPARISON OF 87DIA4 WITH AQA3

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
# LOC	15	13	8	15	14	8	14	8	8	10	13
P VALUE	0.86	0.72	0.34	0.86	0.00**	0.58	0.56	0.64	0.61	0.01*	0.00**

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

A. ORIGIN AND BREEDING HISTORY

Inbred plant 87DIA4 was derived from the cross between a line derived from 2FACC and 3AZA1.

87DIA4 shows uniformity and stability within the limits of environmental influence for the traits described hereinafter in Table 4. 87DIA4 has been self-pollinated and ear-rowed a sufficient number of generations with careful attention paid to uniformity of plant type to ensure homozygosity and phenotypic stability. No variant traits have been observed or are expected in 87DIA4.

A deposit of 2500 seeds of plant designated 87DIA4 has been made with the American Type Culture Collection (ATCC), Rockville Pike, Bethesda, Md. on Sep. 11, 1998. Those deposited seeds have been assigned Accession No. 203192. The deposit was made in accordance with the terms and provisions of the Budapest Treaty relating to deposit of microorganisms and is made for a term of at least thirty (30) years and at least five (05) years after the most recent request for the furnishing of a sample of the deposit was received by the depository, or for the effective term of the patent, whichever is longer, and will be replaced if it becomes non-viable during that period.

Inbred corn plants can be reproduced by planting such inbred seeds, growing the resulting corn plants under self-pollinating or sib-pollinating conditions with adequate isolation using standard techniques well known to an artisan skilled in the agricultural arts. Seeds can be harvested from such a plant using standard, well known procedures.

The origin and breeding history of inbred plant 87DIA4 can be summarized as follows:

Summer 1988 The cross 2FACC and AQA3 was made. Both inbreds are proprietary to DEKALB Genetics Corporation.

Winter 1988 S0 seed was grown (nursery row 67-51).

Summer 1989 S1 seed was grown (nursery rows 4-25 to 4-38).

Winter 1989 S2 seed was grown ear-to-row (nursery row 649-62).

Summer 1990 S3 seed was grown ear-to-row (nursery row 130-15).

Winter 1990 S4 seed was grown ear-to-row (nursery row C23-23).

Summer 1991 S5 seed was grown ear-to-row (nursery row 222-67).

Summer 1992 S6 seed was grown ear-to-row (nursery row 418-56).

Summer 1993 S7 seed was grown ear-to-row (nursery rows 346-32 to 346-39). Seed from all rows was bulked to form 87DIA4.

B. PHENOTYPIC DESCRIPTION

In accordance with another aspect of the present invention, there is provided a corn plant having the physiological and morphological characteristics of corn plant 87DIA4. A description of the physiological and morphological characteristics of corn plant 87DIA114 is presented in Table 4.

TABLE 4

MORPHOLOGICAL TRAITS FOR THE 87DIA4 PHENOTYPE

CHARACTERISTIC	87DIA4	2FACC	3AZA1	AQA3
1. STALK				
Diameter (width) cm	1.9	2.2	2.0	2.2
Anthocyanin	Absent	Absent	Absent	Absent
Nodes with Brace Roots	1.4	1.9	2.0	1.5
Brace Root Color	Red	Purple	—	Green
Internode Direction	Straight	Straight	Straight	Straight

TABLE 4-continued

MORPHOLOGICAL TRAITS FOR THE 87DIA4 PHENOTYPE

CHARACTERISTIC	87DIA4	2FACC	3AZA1	AQA3
Internode Length cm.	10.2	12.7	14.0	11.3
2. LEAF				
Color	Med Green	Med Green	Med Green	Med Green
Length cm.	68.0	71.1	66.2	67.9
Width cm.	10.0	8.7	7.9	8.3
Sheath Anthocyanin	Weak	Weak	Weak	Absent
Sheath Pubescence	Medium	Light	Medium	Medium
Marginal Waves	Few	Few	Few	Few
Longitudinal Creases	Absent	Absent	—	Few
3. TASSEL				
Attitude	Compact	Compact	—	Open
Length cm.	29.5	26.7	33.0	33.0
Spike Length cm.	19.5	19.1	24.4	23.1
Peduncle Length cm.	2.9	5.2	3.6	3.6
Branch Number	4.5	7.7	3.8	5.5
Anther Color	Red	Pink	Tan	Grn-Yellow
Glume Color	Green	Green	Green	Green
Glume Band	Absent	Absent	Absent	Absent
4. EAR				
Silk Color	Pink	Tan	Grn-Yellow	Grn-Yellow
Number Per Stalk	1.1	1.1	1.6	1.4
Position (attitude)	Upright	Upright	Pendant	Upright
Length cm.	15.6	13.9	16.4	16.3
Shape	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Diameter cm.	3.8	4.2	3.4	3.6
Weight gm.	99.1	116.3	89.8	93.1
Shank Length cm.	16.5	14.8	20.7	14.9
Husk Bract	Short	Short	Short	Short
Husk Cover cm.	3.4	6.6	1.9	3.2
Husk Opening	Tight	Intermediate	—	Intermediate
Husk Color Fresh	Green	Lt Green	Green	Lt Green
Husk Color Dry	Buff	Buff	Buff	Buff
Cob Diameter cm.	2.3	2.6	1.7	2.1
Cob Color	Red	Red	Red	Red
Shelling Percent	85.1	81.4	85.8	85.0
5. KERNEL				
Row Number	14.0	14.7	12.3	15.1
Number Per row	31.4	25.5	32.2	33.2
Row Direction	Curved	Curved	Curved	Curved
Type	Dent	Dent	Dent	Dent
Cap Color	Yellow	Yellow	Yellow	Lemon Yellow
Side Color	Yellow	Deep Yellow	Orange	Orange
Length (depth) mm.	10.2	10.7	9.2	9.6
Width mm.	8.1	8.1	7.3	7.1
Thickness	4.3	4.3	4.2	3.8
Weight of 1000K gm.	281.5	280.7	223.8	173.7
Endosperm Type	Normal	Normal	Normal	Normal
Endosperm Color	Yellow	Yellow	Yellow	Yellow

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

IV. ADDITIONAL INBRED CORN PLANTS

The inbred corn plant 171KI3 has been employed with the corn plant of the present invention in order to produce an exemplary hybrid. A description of the physiological and morphological characteristics of this corn plant is presented herein at Table 5. Additional information for this inbred corn plant is presented in co-pending U.S. patent application Ser. No. 08/795,403, filed Feb. 5, 1997, the disclosure of which application is specifically incorporated herein by reference.

TABLE 5

MORPHOLOGICAL TRAITS FOR THE 171KI3 PHENOTYPE

CHARACTERISTIC	171KI3	01CSI2	01IBH2	3IIH6
1. STALK				
Diameter (width) cm.	2.2	2.4	2.1	2.3

TABLE 5-continued

MORPHOLOGICAL TRAITS FOR THE 171KI3 PHENOTYPE				
CHARACTERISTIC	171KI3	01CSI2	01IBH2	3IIH6
Anthocyanin	Absent	Absent	Absent	Absent
Nodes With Brace Roots	0.9	1.8	1.1	0.7
Brace Root Color	Green	Green	Green	—
Internode Direction	Straight	Straight	Straight	Straight
Internode Length cm.	15.9	12.8	14.4	13.1
2. LEAF				
Color	Med Green	—	Med Green	Med Green
Width cm.	9.7	8.9	8.9	8.0
Marginal Waves	Few	Few	Few	Few
3. TASSEL				
Length cm.	42.6	31.2	33.6	35.3
Spike Length cm.	22.9	23.2	23.1	25.2
Peduncle Length cm.	9.6	3.9	8.2	7.6
Branch Number	9.1	7.4	7.8	12.9
Anther Color	Purple	Grn-Yellow	Grn-Yellow	—
Glume Color	Purple	Green	Green	—
Glume Band	Present	Absent	Absent	Absent
4. EAR	Pink	Pink	—	Red
Silk Color	1.0	1.0	1.0	1.4
Number Per Stalk	Upright	—	—	Upright
Position (attitude)	14.6	16.0	14.6	15.6
Length cm.	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Shape	4.0	3.8	4.0	3.9
Diameter cm.	104.9	100.6	103.2	107.6
Weight gm.	10.3	14.1	10.1	9.6
Shank Length cm.	Short	Short	Short	Short
Husk Bract	6.4	2.5	4.4	3.7
Husk Cover cm.	Green	Green	Green	Green
Husk Color Fresh	Buff	Buff	Buff	Buff
Husk Color Dry	2.3	2.4	2.3	2.1
Cob Diameter cm.	Red	—	Red	Red
Cob Color	87.7	80.6	89.0	83.3
Shelling Percent				
5. KERNEL	14.6	14.8	16.3	15.3
Row Number	32.1	27.1	29.3	29.7
Number Per Row	Curved	Curved	Curved	Curved
Row Direction	Dent	—	Dent	—
Type	Yellow	Yellow	Yellow	Yellow
Cap Color	Deep Yellow	—	Orange	
Side Color	11.1	9.4	10.9	10.3
Length (depth) mm.	7.8	8.0	7.4	7.8
Width mm.	3.9	5.2	4.4	4.2
Thickness	269.0	252.4	233.0	247.8
Weight of 1000K gm.	Normal	Normal	Normal	Normal
Endosperm Type	Yellow	Yellow	Yellow	Yellow
Endosperm Color				

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

V. SINGLE GENE CONVERSIONS

When the term inbred corn plant is used in the context of the present invention, this also includes any single gene conversions of that inbred. The term single gene converted plant as used herein refers to those corn plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single gene transferred into the inbred via the backcrossing technique. Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the inbred. The term backcrossing as used herein refers to the repeated crossing of a hybrid progeny back to one of the parental corn plants for that inbred. The parental corn plant which contributes the gene for the desired characteristic is termed the nonrecurrent or donor parent. This terminology refers to the fact that the nonrecurrent parent is used one time in the backcross protocol and therefore does not recur. The parental corn plant to which the gene or genes from the nonrecurrent parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol (Poehlman & Sleper, 1994; Fehr, 1987). In a typical backcross protocol, the original inbred of interest (recurrent parent) is crossed to a second inbred (nonrecurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a corn plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the

converted plant, in addition to the single transferred gene from the nonrecurrent parent.

The selection of a suitable recurrent parent is an important step for a successful backcrossing procedure. The goal of a backcross protocol is to alter or substitute a single trait or characteristic in the original inbred. To accomplish this, a single gene of the recurrent inbred is modified or substituted with the desired gene from the nonrecurrent parent, while retaining essentially all of the rest of the desired genetic, and therefore the desired physiological and morphological, constitution of the original inbred. The choice of the particular nonrecurrent parent will depend on the purpose of the backcross, one of the major purposes is to add some commercially desirable, agronomically important trait to the plant. The exact backcrossing protocol will depend on the characteristic or trait being altered to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele may also be transferred. In this instance it may be necessary to introduce a test of the progeny to determine if the desired characteristic has been successfully transferred.

Many single gene traits have been identified that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques. Single gene traits may or may not be transgenic, examples of these traits include but are not limited to, male sterility, waxy starch, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, male fertility, enhanced nutritional quality, industrial usage, yield stability and yield enhancement. These genes are generally inherited through the nucleus. Some known exceptions to this are the genes for male sterility, some of which are inherited cytoplasmically, but still act as single gene traits. Several of these single gene traits are described in U.S. Ser. No. 07/113,561, filed Aug. 25, 1993, the disclosure of which is specifically hereby incorporated by reference.

Direct selection may be applied where the single gene acts as a dominant trait. An example might be the herbicide resistance trait. For this selection process, the progeny of the initial cross are sprayed with the herbicide prior to the backcrossing. The spraying eliminates any plants which do not have the desired herbicide resistance characteristic, and only those plants which have the herbicide resistance gene are used in the subsequent backcross. This process is then repeated for all additional backcross generations.

The waxy characteristic is an example of a recessive trait. In this example, the progeny resulting from the first backcross generation (BC1) must be grown and selfed. A test is then run on the selfed seed from the BC1 plant to determine which BC1 plants carried the recessive gene for the waxy trait. In other recessive traits, additional progeny testing, for example growing additional generations such as the BC1S1 may be required to determine which plants carry the recessive gene.

VI. ORIGIN AND BREEDING HISTORY OF AN EXEMPLARY SINGLE GENE CONVERTED PLANT

85DGD1 MLms is a single gene conversion of 85DGD1 to cytoplasmic male sterility. 85DGD1 MLms was derived using backcross methods. 85DGD1 (a proprietary inbred of DEKALB Genetics Corporation) was used as the recurrent parent and MLms, a germplasm source carrying ML cytoplasmic sterility, was used as the nonrecurrent parent. The breeding history of the single gene converted inbred 85DGD1 MLms can be summarized as follows:

Hawaii Nurseries Planting Date Apr. 2, 1992 Made up S-O: Female row 585 male row 500

Hawaii Nurseries Planting Date Jul. 15, 1992 S-O was grown and plants were backcrossed times 85DGD1 (rows 444' 443)

Hawaii Nurseries Planting Date Bulked seed of the BC1 was grown and Nov. 18, 1992 backcrossed times 85DGD1 (rows V3-27' V3-26)

Hawaii Nurseries Planting Date Apr. 2, 1993 Bulked seed of the BC2 was gown and backcrossed times 85DGD1 (rows 37' 36)

Hawaii Nurseries Planting Date Jul. 14, 1993 Bulked seed of the BC3 was grown and backcrossed times 85DGD1 (rows 99' 98)

Hawaii Nurseries Planting Date Bulked seed of BC4 was grown and backcrossed Oct. 28, 1993 times 85DGD1 (rows KS-63' KS-62)

Summer 1994 A single ear of the BC5 was grown and backcrossed times 85DGD1 (MC94-822' MC94-822-7)

Winter 1994 Bulked seed of the BC6 was grown and backcrossed times 85DGD1 (3Q-1' 3Q-2)

Summer 1995 Seed of the BC7 was bulked and named 85DGD1 MLms.

VII. TISSUE CULTURE AND IN VITRO REGENERATION OF CORN PLANTS

A further aspect of the invention relates to tissue culture of corn plants designated 87DIA4. As used herein, the term "tissue culture" indicates a composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant. Exemplary types of tissue cultures are protoplasts, calli, plant clumps, and plant cells that are intact in plants or parts of plants, such as embryos, pollen, flowers, kernels, ears, cobs, leaves, husks, stalks, roots, root tips, anthers, silk and the like. In a preferred embodiment, tissue culture is embryos, protoplast, meristematic cells, pollen, leaves or anthers. Means for preparing and maintaining plant tissue culture are well known in the art. By way of example, a tissue culture comprising organs such as tassels or anthers, has been used to produce regenerated plants. (See, U.S. patent applications Ser. No. 07/992,637, filed Dec. 18, 1992 and 07/995,938, filed Dec. 21, 1992, now issued as U.S. Pat. No. 5,322,789, issued Jun. 21, 1994, the disclosures of which are incorporated herein by reference).

VIII. TASSEL/ANTHER CULTURE

Tassels contain anthers which in turn enclose microspores. Microspores develop into pollen. For anther/microspore culture, if tassels are the plant composition., they are preferably selected at a stage when the microspores are uninucleate, that is, include only one, rather than 2 or 3 nuclei. Methods to determine the correct stage are well known to those killed in the art and include mitramycin fluorescent staining (Pace et al., 1987), trypan blue (preferred) and acetocarmine squashing. The mid-uninucleate microspore stage has been found to be the developmental stage most responsive to the subsequent methods disclosed to ultimately produce plants.

Although microspore-containing plant organs such as tassels can generally be pretreated at any cold temperature below about 25° C., a range of 4 to 25° C. is preferred, and a range of 8 to 14° C. is particularly preferred. Although other temperatures yield embryoids and regenerated plants, cold temperatures produce optimum response rates compared to pretreatment at temperatures outside the preferred range. Response rate is measured as either the number of embryoids or the number of regenerated plants per number of microspores initiated in culture.

Although not required, when tassels are employed as the plant organ, it is generally preferred to sterilize their surface.

Following surface sterilization of the tassels, for example, with a solution of calcium hypochloride, the anthers are removed from about 70 to 150 spikelets (small portions of the tassels) and placed in a preculture or pretreatment medium. Larger or smaller amounts can be used depending on the number of anthers.

When one elects to employ tassels directly, tassels are preferably pretreated at a cold temperature for a predefined time, preferably at 10° C. for about 4 days. After pretreatment of a whole tassel at a cold temperature, dissected anthers are further pretreated in an environment that diverts microspores from their developmental pathway. The function of the preculture medium is to switch the developmental program from one of pollen development that of embryoid/callus development. An embodiment of such an environment in the form of a preculture medium includes a sugar alcohol, for example mannitol or sorbitol, inositol or the like. An exemplary synergistic combination is the use of mannitol at a temperature of about 10° C. for a period ranging from about 10 to 14 days. In a preferred embodiment, 3 ml of 0.3 M mannitol combined with 50 mg/l of ascorbic acid, silver nitrate and colchicine is used for incubation of anthers at 10° C. for between 10 and 14 days. Another embodiment is to substitute sorbitol for mannitol. The colchicine produces chromosome doubling at this early stage. The chromosome doubling agent is preferably only present at the preculture stage.

It is believed that the mannitol or other similar carbon structure or environmental stress induces starvation and functions to force microspores to focus their energies on entering developmental stages. The cells are unable to use, for example, mannitol as a carbon source at this stage. It is believed that these treatments confuse the cells causing them to develop as embryoids and plants from microspores. Dramatic increases in development from these haploid cells, as high as 25 embryoids in 10^4 microspores, have resulted from using these methods.

In embodiments where microspores are obtained from anthers, microspores can be released from the anthers into an isolation medium following the mannitol preculture step. One method of release is by disruption of the anthers, for example, by chopping the anthers into pieces with a sharp instrument, such as a razor blade, scalpel or Waring blender. The resulting mixture of released microspores, anther fragments and isolation medium are then passed through a filter to separate microspores from anther wall fragments. An embodiment of a filter is a mesh, more specifically, a nylon mesh of about 112 mm pore size. The filtrate which results from filtering the microspore-containing solution is preferably relatively free of anther fragments, cell walls and other debris.

In a preferred embodiment, isolation of microspores is accomplished at a temperature below about 25° C. and, preferably at a temperature of less than about 15° C. Preferably, the isolation media, dispersing tool (e.g., razor blade) funnels, centrifuge tubes and dispersing container (e.g., petri dish) are all maintained at the reduced temperature during isolation. The use of a precooled dispersing tool to isolate maize microspores has been reported (Gaillard et al., 1991).

Where appropriate and desired, the anther filtrate is then washed several times in isolation medium. The purpose of the washing and centrifugation is to eliminate any toxic compounds which are contained in the non-microspore part of the filtrate and are created by the chopping process. The centrifugation is usually done at decreasing spin speeds, for example, 1000, 750, and finally 500 rpms.

The result of the foregoing steps is the preparation of a relatively pure tissue culture suspension of microspores that are relatively free of debris and anther remnants.

To isolate microspores, an isolation media is preferred. An isolation media is used to separate microspores from the anther walls while maintaining their viability and embryogenic potential. An illustrative embodiment of an isolation media includes a 6 percent sucrose or maltose solution combined with an antioxidant such as 50 mg/l of ascorbic acid, 0.1 mg/l biotin and 400 mg/l of proline, combined with 10 mg/l of nicotinic acid and 0.5 mg/l $AgNO_3$. In another embodiment, the biotin and proline are omitted.

An isolation media preferably has a higher antioxidant level where used to isolate microspores from a donor plant (a plant from which a plant composition containing a microspore is obtained) that is field grown in contrast to greenhouse grown. A preferred level of ascorbic acid in an isolation medium is from about 50 mg/l to about 125 mg/l and, more preferably from about 50 mg/l to about 100 mg/l.

One can find particular benefit in employing a support for the microspores during culturing and subculturing. Any support that maintains the cells near the surface can be used. The microspore suspension is layered onto a support, for example by pipetting. There are several types of supports which are suitable and are within the scope of the invention. An illustrative embodiment of a solid support is a TRANSWELL® culture dish. Another embodiment of a solid support for development of the microspores is a bilayer plate wherein liquid media is on top of a solid base. Other embodiments include a mesh or a millipore filter. Preferably, a solid support is a nylon mesh in the shape of a raft. A raft is defined as an approximately circular support material which is capable of floating slightly above the bottom of a tissue culture vessel, for example, a petri dish, of about a 60 or 100 mm size, although any other laboratory tissue culture vessel will suffice. In an illustrative embodiment, a raft is about 55 mm in diameter.

Culturing isolated microspores on a solid support, for example, on a 10 mm pore nylon raft floating on 2.2 ml of medium in a 60 mm petri dish, prevents microspores from sinking into the liquid medium and thus avoiding low oxygen tension. These types of cell supports enable the serial transfer of the nylon raft with its associated microspore/embryoids ultimately to full strength medium containing activated charcoal and solidified with, for example, GELRITE™ (solidifying agent). The charcoal is believed to absorb toxic wastes and intermediaries. The solid medium allows embryoids to mature.

The liquid medium passes through the mesh while the microspores are retained and supported at the medium-air interface. The surface tension of the liquid medium in the petri dish causes the raft to float. The liquid is able to pass through the mesh: consequently, the microspores stay on top. The mesh remains on top of the total volume of liquid medium. An advantage of the raft is to permit diffusion of nutrients to the microspores. Use of a raft also permits transfer of the microspores from dish to dish during subsequent subculture with minimal loss, disruption or disturbance of the induced embryoids that are developing. The rafts represent an advantage over the multi-welled TRANSWELL® plates, which are commercially available from COSTAR, in that the commercial plates are expensive. Another disadvantage of these plates is that to achieve the serial transfer of microspores to subsequent media, the membrane support with cells must be peeled off the insert in the wells. This procedure does not produce as good a yield nor as efficient transfers, as when a mesh is used as a vehicle for cell transfer.

The culture vessels can be further defined as either (1) a bilayer 60 mm petri plate wherein the bottom 2 ml of medium are solidified with 0.7 percent agarose, overlaid with 1 mm of liquid containing the microspores; (2) a nylon mesh raft wherein a wafer of nylon is floated on 1.2 ml of medium and 1 ml of isolated microspores is pipetted on top; or (3) TRANSWELL® plates wherein isolated microspores are pipetted onto membrane inserts which support the microspores at the surface of 2 ml of medium.

After the microspores have been isolated, they are cultured in a low strength anther culture medium until about the 50 cell stage when they are subcultured onto an embryoid/callus maturation medium. Medium is defined at this stage as any combination of nutrients that permit the microspores to develop into embryoids or callus. Many examples of suitable embryoid/callus promoting media are well known to those skilled in the art. These media will typically comprise mineral salts, a carbon source, vitamins, growth regulations. A solidifying agent is optional. A preferred embodiment of such a media is referred to by the inventor as the "D medium" which typically includes 6N1 salts, $AgNO_3$ and sucrose or maltose.

In an illustrative embodiment, 1 ml of isolated microspores are pipetted onto a 10 mm nylon raft and the raft is floated on 1.2 ml of medium "D", containing sucrose or, preferably maltose. Both calli and embryoids can develop. Calli are undifferentiated aggregates of cells. Type I is a relatively compact, organized and slow growing callus. Type II is a soft, friable and fast-growing one. Embryoids are aggregates exhibiting some embryo-like structures. The embryoids are preferred for subsequent steps to regenerating plants. Culture medium "D" is an embodiment of medium that follows the isolation medium and replaces it. Medium "D" promotes growth to an embryoid/callus. This medium comprises 6N1 salts at ⅛ the strength of a basic stock solution, (major components) and minor components, plus 12 percent sucrose or, preferably 12 percent maltose, 0.1 mg/l B1, 0.5 mg/l nicotinic acid, 400 mg/l proline and 0.5 mg/l silver nitrate. Silver nitrate is believed to act as an inhibitor to the action of ethylene. Multi-cellular structures of approximately 50 cells each generally arise during a period of 12 days to 3 weeks. Serial transfer after a two week incubation period is preferred.

After the petri dish has been incubated for an appropriate period of time, preferably two weeks, in the dark at a predefined temperature, a raft bearing the dividing microspores is transferred serially to solid based media which promotes embryo maturation. In an illustrative embodiment, the incubation temperature is 30° C. and the mesh raft supporting the embryoids is transferred to a 100 mm petri dish containing the 6N1-TGR-4P medium, an "anther culture medium." This medium contains 6N1 salts, supplemented with 0.1 mg/l TIBA, 12 percent sugar (sucrose, maltose or a combination thereof), 0.5 percent activated charcoal, 400 mg/l proline, 0.5 mg/l B, 0.5 mg/l nicotinic acid, and 0.2 percent GELRITE™ (solidifying agent) and is capable of promoting the maturation of the embryoids. Higher quality embryoids, that is, embryoids which exhibit more organized development, such as better shoot meristem formation without precocious germination were typically obtained with the transfer to full strength medium compared to those resulting from continuous culture using only, for example, the isolated microspore culture (IMC) Medium "D." The maturation process permits the pollen embryoids to develop further in route toward the eventual regeneration of plants. Serial transfer occurs to full strength solidified 6N1 medium using either the nylon raft, the TRANSWELL® membrane or bilayer plates, each one requiring the movement of developing embryoids to permit further development into physiologically more mature structures.

In an especially preferred embodiment, microspores are isolated in an isolation media comprising about 6 percent maltose, cultured for about two weeks in an embryoid/calli induction medium comprising about 12 percent maltose and then transferred to a solid medium comprising about 12 percent sucrose.

At the point of transfer of the raft after about two weeks incubation, embryoids exist on a nylon support. The purpose of transferring the raft with the embryoids to a solidified medium after the incubation is to facilitate embryo maturation. Mature embryoids at this point are selected by visual inspection indicated by zygotic embryo-like dimensions and structures and are transferred to the shoot initiation medium. It is preferred that shoots develop before roots, or that shoots and roots develop concurrently. If roots develop before shoots, plant regeneration can be impaired. To produce solidified media, the bottom of a petri dish of approximately 100 mm is covered with about 30 ml of 0.2 percent GELRITE™ (solidifying agent) solidified medium. A sequence of regeneration media are used for whole plant formation from the embryoids.

During the regeneration process, individual embryoids are induced to form plantlets. The number of different media in the sequence can vary depending on the specific protocol used. Finally, a rooting medium is used as a prelude to transplanting to soil. When plantlets reach a height of about 5 cm, they are then transferred to pots for further growth into flowering plants in a greenhouse by methods well known to those skilled in the art.

Plants have been produced from isolated microspore cultures by methods disclosed herein, including self-pollinated plants. The rate of embryoid induction was much higher with the synergistic preculture treatment consisting of a combination of stress factors, including a carbon source which can be capable of inducing starvation, a cold temperature and colchicine, than has previously been reported. An illustrative embodiment of the synergistic combination of treatments leading to the dramatically improved response rate compared to prior methods, is a temperature of about 10° C., mannitol as a carbon source, and 0.05 percent colchicine.

The inclusion of ascorbic acid, an anti-oxidant, in the isolation medium is preferred for maintaining good microspore viability. However, there seems to be no advantage to including mineral salts in the isolation medium. The osmotic potential of the isolation medium was maintained optimally with about 6 percent sucrose, although a range of 2 percent to 12 percent is within the scope of this invention.

In an embodiment of the embryoid/callus organizing media, mineral salts concentration in IMC Culture Media "D" is (⅛x), the concentration which is used also in anther culture medium. The 6N1 salts major components have been modified to remove ammonium nitrogen. Osmotic potential in the culture medium is maintained with about 12 percent sucrose and about 400 mg/l proline. Silver nitrate (0.5 mg/l) was included in the medium to modify ethylene activity. The preculture media is further characterized by having a pH of about 5.7 to 6.0. Silver nitrate and vitamins do not appear to be crucial to this medium but do improve the efficiency of the response.

Whole anther cultures can also be used in the production of monocotyledonous plants from a plant culture system. There are some basic similarities of anther culture methods

and microspore culture methods with regard to the media used. A difference from isolated microspore cultures is that undisrupted anthers are cultured, so that a support, eg., a nylon mesh support, is not needed. The first step in developing the anther cultures is to incubate tassels at a cold temperature. A cold temperature is defined as less than about 25° C. More specifically, the incubation of the tassels is preferably performed at about 10° C. A range of 8 to 14° C. is also within the scope of the invention. The anthers are then dissected from the tassels, preferably after surface sterilization using forceps, and placed on solidified medium. An example of such a medium is designated by the inventors as 6N1-TGR-P4.

The anthers are then treated with environmental conditions that area combinations of stresses that are capable of diverting microspores from gametogenesis to embryogenesis. It is believed that the stress effect of sugar alcohols in the preculture medium, for example, mannitol, is produced by inducing starvation at the predefined temperature. In one embodiment, the incubation pretreatment is for about 14 days at 10° C. It was found that treating the anthers in addition with a carbon structure, an illustrative embodiment being a sugar alcohol, preferably, mannitol, produces dramatically higher anther culture response rates as measured by the number of eventually regenerated plants, than by treatment with either cold treatment or mannitol alone. These results are particularly surprising in light of teachings that cold is better than mannitol for these purposes, and that warmer temperatures interact with mannitol better.

To incubate the anthers, they are floated on a preculture medium which diverts the microspores from gametogenesis, preferably on a mannitol carbon structure,, more specifically, 0.3 M of mannitol plus 50 mg/l of ascorbic acid. 3 ml is about the total amount in a dish, for example, a tissue culture dish, more specifically, a 60 mm petri dish. Anthers are isolated from about 120 spikelets for one dish yields about 360 anthers.

Chromosome doubling agents can be used in the preculture media for anther cultures. Several techniques for doubling chromosome number (Jensen, 1974; Wan et al., 1989) have been described. Colchicine is one of the doubling agents. However, developmental abnormalities arising from in vitro cloning are further enhanced by colchicine treatments, and previous reports indicated that colchicine is toxic to microspores. The addition of colchicine in increasing concentrations during mannitol pretreatment prior to anther culture and microspore culture has achieved improved percentages.

An illustrative embodiment of the combination of a chromosome doubling agent and preculture medium is one which contains colchicine. In a specific embodiment, the colchicine level is preferably about 0.05 percent. The anthers remain in the mannitol preculture medium with the additives for about 10 days at 10° C. Anthers are then placed on maturation media, for example, that designated 6N1-TGR-P4, for 3 to 6 weeks to induce embryoids. If the plants are to be regenerated from the embryoids, shoot regeneration medium is employed, as in the isolated microspore procedure described in the previous sections. Other regeneration media can be used sequentially to complete regeneration of whole plants.

The anthers are then exposed to embryoid/callus promoting medium, for example, that designated 6N1-TGR-P4 to obtain callus or embryoids. The embryoids are recognized by identification visually of embryonic-like structures. At this stage, the embryoids are transferred serially to a series of regeneration media. In an illustrative embodiment, :he shoot initiation medium comprises BAP (6-benzyl-aminopurine) and NAA (naphthalene acetic acid). Regeneration protocols for isolated microspore cultures and anther cultures are similar.

IX. OTHER CULTURES AND REGENERATION

The present invention contemplates a corn plant regenerated from a tissue culture of an inbred (e.g., 87DIA4) or hybrid plant (e.g., 4033843) of the present invention. As is well known in the art, tissue culture of corn can be used for the in vitro regeneration of a corn plant. By way of example, a process of tissue culturing and regeneration of corn is described in European Patent Application, publication 160, 390, the disclosure of which is incorporated by reference. Corn tissue culture procedures are also described in Green & Rhodes (1982) and Duncan et al., (1985). The study by Duncan et al. (1985) indicates that 97 percent of cultured plants produced calli capable of regenerating plants. Subsequent studies have shown that both inbreds and hybrids produced 91 percent regenerable calli that produced plants.

Other studies indicate that non-traditional tissues are capable of producing somatic embryogenesis and plant regeneration. See, e.g., Songstad et al. (1988); Rao et al. (1986); and Conger et al. (1987), the disclosures of which are incorporated herein by reference. Regenerable cultures may be initiated from immature embryos as described in PCT publication WO 95/06128, the disclosure of which is incorporated herein by reference.

Briefly, by way of example, to regenerate a plant of this invention, cells are selected following growth in culture. Where employed, cultured cells are preferably grown either on solid supports or in the form of liquid suspensions as set forth above. In either instance, nutrients are provided to the cells in the form of media, and environmental conditions are controlled. There are many types of tissue culture media comprising amino acids, salts, sugars, hormones and vitamins. Most of the media employed to regenerate inbred and hybrid plants have some similar components, the media differ in the composition and proportions of their ingredients depending on the particular application envisioned. For example, various cell types usually grow in more than one type of media, but exhibit different growth rates and different morphologies, depending on the growth media. In some media, cells survive but do not divide. Various types of media suitable for culture of plant cells have been previously described and discussed above.

An exemplary embodiment for culturing recipient corn cells in suspension cultures includes using embryogenic cells in Type II (Armstrong & Green, 1985; (Gordon-Kamm et al., 1990) callus, selecting for small (10 to 30 m) isodiametric, cytoplasmically dense cells, growing the cells in suspension cultures with hormone containing media, subculturing into a progression of media to facilitate development of shoots and roots, and finally, hardening the plant and readying it metabolically for growth in soil.

Meristematic cells (i.e., plant cells capable of continual cell division and characterized by an undifferentiated cytological appearance, normally found at growing points or tissues in plants such as root tips, stem apices, lateral buds, etc.) can be cultured.

Embryogenic calli are produced essentially as described in PCT Publication WO 95/06128. Specifically, inbred plants or plants from hybrids produced from crossing an inbred of the present invention with another inbred are grown to flowering in a greenhouse. Explants from at least one of the following F_1 tissues: the immature tassel tissue, intercalary meristems and leaf bases, apical meristems, immature ears and immature embryos are placed in an

initiation medium which contain MS salts, supplemented with thiamine, agar, and sucrose. Cultures are incubated in the dark at about 23° C. All culture manipulations and selections are performed with the aid of a dissecting microscope.

After about 5 to 7 days, cellular outgrowths are observed from the surface of the explants. After about 7 to 21 days, the outgrowths are subcultured by placing them into fresh medium of the same composition. Some of the intact immature embryo explants are placed on fresh medium. Several subcultures later (after about 2 to 3 months) enough material is present from explants for subdivision of these embryogenic calli into two or more pieces.

Callus pieces from different explants are not mixed. After further growth and subculture (about 6 months after embryogenic callus initiation), there are usually between 1 and 100 pieces derived ultimately from each selected explant. During this time of culture expansion, a characteristic embryogenic culture morphology develops as a result of careful selection at each subculture. Any organized structures resembling roots or root primordia are discarded. Material known from experience to lack the capacity for sustained growth is also discarded (translucent, watery, embryogenic structures). Structures with a firm consistency resembling at least in part the scutelum of the in vivo embryo are selected.

The callus is maintained on agar-solidified MS or N6-type media. A preferred hormone is 2,4-D. A second preferred hormone is dicamba. Visual selection of embryo-like structures is done to obtain subcultures. Transfer of material other than that displaying embryogenic morphology results in loss of the ability to recover whole plants from the callus.

Cell suspensions are prepared from the calli by selecting cell populations that appear homogeneous macroscopically. A portion of the friable, rapidly growing embryogenic calli is inoculated into MS or N6 Medium containing 2,4-D or dicamba. The calli in medium are incubated at about 27° C. on a gyrotary shaker in the dark or in the presence of low light. The resultant suspension culture is transferred about once every three to seven days, preferably every three to four days, by taking about 5 to 10 ml of the culture and introducing this inoculum into fresh medium of the composition listed above.

For regeneration, embryos which appear on the callus surface are selected and regenerated into whole plants by transferring the embryogenic structure, into a sequence of solidified media which include decreasing concentrations of 2,4-D or other auxins. Other hormones which can be used in culture media include dicamba, NAA, ABA, BAP, and 2-NCA. The reduction is relative to the concentration used in culture maintenance media. Plantlets are regenerated from these embryos by transfer to a hormone-free medium, subsequently transferred to soil, and grown to maturity.

Progeny are produced by taking pollen and selfing, backcrossing or sibling regenerated plants by methods well known to those skilled in the arts. Seeds are collected from the regenerated plants.

X. PROCESSES OF PREPARING CORN PLANTS AND THE CORN PLANTS PRODUCED BY SUCH CROSSES

The present invention also provides a process of preparing a novel corn plant and a corn plant produced by such a process. In accordance with such a process, a first parent corn plant is crossed with a second parent corn plant wherein at least one of the first and second corn plants is the inbred corn plant 87DIA4. In one embodiment, a corn plant prepared by such a process is a first generation F_1 hybrid corn plant prepared by a process wherein both the first and second parent corn plants are inbred corn plants.

Corn plants (*Zea mays L.*) can be crossed by either natural or mechanical techniques. Natural pollination occurs in corn when wind blows pollen from the tassels to the silks that protrude from the tops of the incipient ears. Mechanical pollination can be effected either by controlling the types of pollen that can blow onto the silks or by pollinating by hand.

In a preferred embodiment, crossing comprises the steps of:

(a) planting in pollinating proximity seeds of a first and a second parent corn plant, and preferably, seeds of a first inbred corn plant and a second, distinct inbred corn plant;

(b) cultivating or growing the seeds of the first and second parent corn plants into plants that bear flowers;

(c) emasculating flowers of either the first or second parent corn plant, i.e., treating the flowers so as to prevent pollen production, in order to produce an emasculated parent corn plant;

(d) allowing natural cross-pollination to occur between the first and second parent corn plant;

(e) harvesting seeds produced on the emasculated parent corn plant; and, where desired,

(f) growing the harvested seed into a corn plant, or preferably, a hybrid corn plant.

Parental plants are planted in pollinating proximity to each other by planting the parental plants in alternating rows, in blocks or in any other convenient planting pattern. Plants of both parental parents are cultivated and allowed to grow until the time of flowering. Advantageously, during this growth stage, plants are in general treated with fertilizer and, or other agricultural chemicals as considered appropriate by the grower.

At the time of flowering, in the event that plant 87DIA4, is employed as the male parent, the tassels of the other parental plant are removed from all plants employed as the female parental plant. The detasseling can be achieved manually but also can be done by machine, if desired.

The plants are then allowed to continue to grow and natural cross-pollination occurs as a result of the action of wind, which is normal in the pollination of grasses, including corn. As a result of the emasculation of the female parent plant, all the pollen from the male parent plant 87DIA4 is available for pollination because tassels, and thereby pollen bearing flowering parts, have been previously removed from all plants of the inbred plant being used as the female in the hybridization. Of course, during this hybridization procedure, the parental varieties are grown such that they are isolated from other corn fields to minimize or prevent my accidental contamination of pollen from foreign sources. These isolation techniques are well within the skill of those skilled in this art.

Both parental inbred plants of corn may be allowed to continue to grow until maturity or the male rows may be destroyed after flowering is complete. Only the ears from the female inbred parental plants are harvested to obtain seeds of a novel F_1 hybrid. The novel F_1 hybrid seed produced can then be planted in a subsequent growing season with the desirable characteristics in terms of F_1 hybrid corn plants providing improved grain yields and the other desirable characteristics disclosed herein, being achieved.

Alternatively, in another embodiment, both first and second parent corn plants can come from the same inbred corn plant, i.e., from the inbred designated 87DIA4. Thus, any corn plant produced using a process of the present invention and inbred corn plant 87DIA4, is contemplated by this invention. As used herein, crossing can mean selfing,

backcrossing, crossing to another or the same inbred, crossing to populations, and the like. All corn plants produced using the present inbred corn plant 87DIA4 as a parent are within the scope of this invention.

The utility of the inbred plant 87DIA4 also extends to crosses with other species. Commonly, suitable species will be of the family Graminaceae, and especially of the genera Zea, Tripsacum, Coix, Schlerachne, Polytoca, Chionachne, and Trilobachne, of the tribe Maydeae. Of these, Zea and Tripsacum, are most preferred. Potentially suitable for crosses with 87DIA4 can be the various varieties of grain sorghum, *Sorghum bicolor* (L.) Moench.

A. F_1 HYBRID CORN PLANT AND SEED PRODUCTION

Where the inbred corn plant 87DIA4 is crossed with another, different, corn inbred, a first generation (F_1) corn hybrid plant is produced. Both a F_1 hybrid corn plant and a seed of that F_1 hybrid corn plant are contemplated as aspects of the present invention.

Inbred 87DIA4 has been used to prepare an F_1 hybrid corn plant, designated 4033843.

The goal of a process of producing an F_1 hybrid is to manipulate the genetic complement of corn to generate new combinations of genes which interact to yield new, or improved traits (phenotypic characteristics). A process of producing an F_1 hybrid typically begins with the production of one or more inbred plants. Those plants are produced by repeated crossing of ancestrally related corn plants to try and concentrate certain genes within the inbred plants. The production of inbred 87DIA4 has been set forth hereinbefore.

Corn has a diploid phase which means two conditions of a gene (two alleles) occupy each locus (position on a chromosome). If the alleles are the same at a locus, there is said to be homozygosity. If they are different, there is said to be heterozygosity. In a completely inbred plant, all loci are homozygous. Because many loci when homozygous are deleterious to the plant, in particular leading to reduced vigor, less kernels, weak and/or poor growth, production of inbred plants is an unpredictable and arduous process. Under some conditions, heterozygous advantage at some loci effectively bars perpetuation of homozygosity.

Inbreeding requires coddling and sophisticated manipulation by human breeders. Even in the extremely unlikely event inbreeding rather than crossbreeding occurred in natural corn, achievement of complete inbreeding cannot be expected in nature due to well known deleterious effects of homozygosity and the large number of generations the plant would have to breed in isolation. The reason for the breeder to create inbred plants is to have a known reservoir of genes whose gametic transmission is at least somewhat predictable.

The development of inbred plants generally requires at least about 5 to 7 generations of selfing. Inbred plants are then cross-bred in an attempt to develop improved F_1 hybrids. Hybrids are then screened and evaluated in small scale field trials. Typically, about 10 to 15 phenotypic traits, selected for their potential commercial value, are measured. A selection index of the most commercially important traits is used to help evaluate hybrids. FACT, an acronym for Field Analysis Comparison Trial (strip trials), is an on-farm testing program employed by DEKALB Plant Genetics to perform the final evaluation of the commercial potential of a product.

During the next several years, a progressive elimination of hybrids occurs based on more detailed evaluation of their phenotype. Eventually, strip trials (FACT) are conducted to formally compare the experimental hybrids being developed with other hybrids, some of which were previously developed and generally are commercially successful. That is, comparisons of experimental hybrids are made to competitive hybrids to determine if there was any advantage to further commercial development of the experimental hybrids. Examples of such comparisons are presented in Section B, hereinbelow.

When the inbred parental plant 87DIA4 is crossed with another inbred plant to yield a hybrid (such as the hybrid 4033843), the original inbred can serve as either the maternal or paternal plant. For many crosses, the outcome is the same regardless of the assigned sex of the parental plants.

However, there is often one of the parental plants that is preferred as the maternal plant because of increased seed yield and production characteristics. Some plants produce tighter ear husks leading to more loss, for example due to rot. There can be delays in silk formation which deleteriously affect timing of the reproductive cycle for a pair of parental inbreds. Seed coat characteristics can be preferable in one plant. Pollen can be shed better by one plant. Other variables can also affect preferred sexual assignment of a particular cross.

B. F_1 HYBRID COMPARISONS

As mentioned in Section A, hybrids are progressively eliminated following detailed evaluations of their phenotype, including formal comparisons with other commercially successful hybrids. Strip trials are used to compare the phenotypes of hybrids grown in as many environments as possible. They are performed in many environments to assess overall performance of the new hybrids and to select optimum growing conditions. Because the corn is grown in close proximity, environmental factors that affect gene expression, such as moisture, temperature, sunlight and pests, are minimized. For a decision to be made that a hybrid is worth making commercially available, it is not necessary that the hybrid be better than all other hybrids. Rather, significant improvements must be shown in at least some, traits that would create improvements in some niches.

Examples of such comparative data are set forth hereinbelow in Table 6, which presents a comparison of performance data for the hybrid 4033843, a hybrid made with 87DIA4 as one parent, versus a selected hybrid of commercial value (DK442).

All the data in Table 6 represents results across years and locations for research and/or strip trials. The "NTEST" represents the number of paired observations in designated tests at locations around the United States.

TABLE 6

COMPARATIVE DATA FOR 4033843

HYBRID	NTEST	SI %C	YLD BU	MST PTS	STL %	RTL %	DRP %	FLSTD %M	SV RAT

TABLE 6-continued

COMPARATIVE DATA FOR 4033843

4033843	R 93	110.3	156.3	19.9	5.2	1.4	0.1	101.0	4.1
DK442		99.0	147.7	19.8	7.1	5.2	0.1	100.9	4.1
DEV		11.3**	8.6**	0.1	-1.9**	-3.8**	0.0	0.2	-0.1

HYBRID	NTEST	ELSTD % M	PHT INCH	EHT INCH	BAR %	SG RAT	TST LBS	FGDU	ESTR DAYS
4033843	R 93	104.4	89.8	40.9		5.0	54.2	1214.0	94.0
DK442		102.9	90.2	43.9		3.1	53.4	1251.0	93.9
DEV		1.5+	-0.4	-3.0**		1.8**	0.8**	-36.9**	0.1

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
LEGEND ABBREVIATIONS:
HYBD = Hybrid
TEST = Research/FACT
SI % C = Selection Index (percent of check)
YLD BU = Yield (bushels/acre)
MST PTS = Moisture
STL % = Stalk Lodging (percent)
RTL % = Root Lodging (percent)
DRP % = Dropped Ears (percent)
FLSTD % M = Final Stand (percent of test mean)
SV RAT = Seedling Vigor Rating
ELSTD % M = Early Stand (percent of test mean)
PHT INCH = Plant Height (inches)
EHT INCH = Ear Height (inches)
BAR % = Barren Plants (percent)
SG RAT = Staygreen Rating
TST LBS = Test Weight (pounds)
FGDU = GDUs to Shed
ESTR DAYS = Estimated Relative Maturity (days)

As can be seen in Table 6, the hybrid 4033843 has significantly higher yield with comparable moisture content when compared to a successful commercial hybrid. Significant differences are also shown in Table 6 for many other traits.

C. PHYSICAL DESCRIPTION OF F_1 HYBRIDS

The present invention also provides F_1 hybrid corn plants derived from the corn plant 87DIA4. Physical characteristics of exemplary hybrids are set forth in Table 7, which concerns 4033843, which has 87DIA4 as one inbred parent. An explanation of terms used in Table 7 can be found in the Definitions, set forth herein above.

TABLE 7

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996

	CHARACTERISTIC	VALUE
1.	STALK	
	Diameter (width) cm	2.6
	Anthocyanin	Absent
	Nodes with Brace Roots	1.5
	Brace Root Color	Red
	Internode Direction	Straight
	Internode Length cm.	16.0
2.	LEAF	
	Color	Med Green
	Length cm.	79.9
	Width cm.	10.7
	Sheath Anthocyanin	Absent
	Sheath Pubescence	Medium
	Marginal Waves	Medium
	Longitudinal Creases	Few

TABLE 7-continued

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996

	CHARACTERISTIC	VALUE
3.	TASSEL	
	Attitude	Compact
	Length cm.	48.1
	Spike Length cm.	27.4
	Peduncle Length cm.	12.1
	Branch Number	7.5
	Anther Color	Red
	Glume Color	Purple
	Glume Band	Absent
4.	EAR	
	Silk Color	Tan
	Number Per Stalk	1.1
	Position (attitude)	Upright
	Length cm.	20.7
	Shape	Semi-conical
	Diameter cm.	4.7
	Weight gm.	222.8
	Shank Length cm.	18.7
	Husk Bract	Short
	Husk Opening	Open
	Husk Color Fresh	Green
	Husk Color Dry	Buff
	Cob Diameter cm.	2.4
	Cob Color	Red
	Shelling Percent	88.6
5.	KERNEL	
	Row Number	15.4
	Number Per row	42.6
	Row Direction	Straight
	Type	Dent

TABLE 7-continued

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996	
CHARACTERISTIC	VALUE
Cap Color	Yellow
Side Color	Deep Yellow
Length (depth) mm.	12.0
Width mm.	7.9
Thickness	4.1
Weight of 1000K gm.	307.0
Endosperm Type	Normal
Endosperm Color	Yellow

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

XI. GENETIC COMPLEMENTS

In another aspect, the present invention provides a genetic complement of a plant of this invention. In one embodiment, therefore, the present invention contemplates an inbred genetic complement of the inbred corn plant designated 87DIA4. In another embodiment, the present invention contemplates a hybrid genetic complement formed by the combination of a haploid genetic complement from 87DIA4 and another haploid genetic complement. Means for determining a genetic complement are well-known in the art.

As used herein, the phrase "genetic complement" means an aggregate of nucleotide sequences, the expression of which sequences defines the phenotype of a corn plant or a cell or tissue of that plant. By way of example, a corn plant is genotyped to determine the array of the inherited markers it possesses. Markers are alleles at a single locus. They are preferably inherited in codominant fashion so that the presence of both alleles at a diploid locus is readily detectable, and they are free of environmental variation, i.e., their heritability is 1. This genotyping is preferably performed on at least one generation of the descendant plant for which the numerical value of the quantitative trait or traits of interest are also determined. The array of single locus genotypes is expressed as a profile of marker alleles, two at each locus. The marker allelic composition of each locus can be either homozygous or heterozygous. Homozygosity is a condition where both alleles at a locus are characterized by the same nucleotide sequence. Heterozygosity refers to different conditions of the gene at a locus. Markers that are used for purposes of this invention include restriction fragment length polymorphisms (RFLPs) and isozymes.

A plant genetic complement can be defined by genetic marker profiles that can be considered "fingerprints" of a genetic complement. For purposes of this invention, markers are preferably distributed evenly throughout the genome to increase the likelihood they will be near a quantitative trait loci (QTL) of interest (e.g., in tomatoes, Helentjaris et al., U.S. Pat. No. 5,385,835, Nienhuis et al., 1987). These profiles are partial projections of a sample of genes. One of the uses of markers in general is to exclude, or alternatively include, potential parents as contributing to offspring.

Phenotypic traits characteristic of the expression of a genetic complement of this invention are distinguishable by electrophoretic separation of DNA sequences cleaved by various restriction endonucleases. Those traits (genetic markers) are termed RFLPs (restriction fragment length polymorphisms).

Restriction fragment length polymorphisms (RFLPs) are genetic differences detectable by DNA fragment lengths, typically revealed by agarose gel electrophoresis, after restriction endonuclease digestion of DNA. There are large numbers of restriction endonucleases available, characterized by their nucleotide cleavage sites and their source, e.g., Eco RI. Variations in RFLPs result from nucleotide base pair differences which alter the cleavage sites of the restriction endonucleases, yielding different sized fragments.

Means for performing RFLP analyses are well known in the art. Restriction fragment length polymorphism analyses reported herein were conducted by Linkage Genetics. This service is available to the public on a contractual basis. Probes were prepared to the fragment sequences, these probes being complementary to the sequences thereby being capable of hybridizing to them under appropriate conditions well known to those ,,killed in the art. These probes were labeled with radioactive isotopes or fluorescent dyes for ease of detection. After the fragments were separated by size, they were identified by the probes. Hybridization with a unique cloned sequence permits the identification of a specific chromosomal region (locus). Because all alleles at a locus are detectable, RFLPs are codominant alleles, thereby satisfying a criteria for a genetic marker. They differ from some other types of markers, e.g., from isozymes, in that they reflect the primary DNA sequence, they are not products of transcription or translation. Furthermore, different RFLP genetic marker profiles result from different arrays of restriction endonucleases.

The RFLP genetic marker profile of each of the parental inbreds and exemplary resultant hybrids were determined. Because an inbred is essentially homozygous at all relevant loci, an inbred should, in almost all cases, have only one allele at each locus. In contrast, a diploid genetic marker profile of a hybrid should be the sum of those parents, e.g., if one inbred parent had the allele A at a particular locus, and the other inbred parent had B, the hybrid is AB by inference. Subsequent generations of progeny produced by selection and breeding are anticipated to be of genotype A, B, or AB for that locus position. When the F1 plant is used to produce an inbred, the locus should be either A or B for that position. Surprisingly, it has been observed that in certain instances, novel RFLP genotypes arise during the breeding process. For example, a genotype of C is observed at a particular locus position from the cross of parental inbreds with A and B at that locus. Such a novel RFLP genotype is observed for the 87DIA4, at least, for the RFLP markers M5213S and M8B2369S, as shown in Table 8. These novel RFLP markers further define the 87DIA4 inbred from the parental inbreds from which it was derived. An RFLP genetic marker profile of 87DIA4 is presented in Table 8.

TABLE 8

RFLP PROFILE OF 87DIA4

PROBE/ENZYME	87DIA4	2FACC	3AZA1	AQA3
M0264H	D	G	G	D
M0306H	A	A	—	A
M0445E	C	B	B	C
M1120S	F	—	D	F
M1234H	D	D	E	E
M1236H	A	A	—	A
M1238H	A	A	F	K
M1401E	C	C	C	A
M1406H	A	—	B	B
M1447H	B	B	E	B
M1B725E	B	B	C	C
M2239H	A	A	C	C
M2297H	A	A	E	A

TABLE 8-continued

RFLP PROFILE OF 87DIA4

PROBE/ENZYME	87DIA4	2FACC	3AZA1	AQA3
M2298E	C	B	C	C
M2402H	E	E	E	E
M3212S	A	A	B	A
M3247E	D	B	D	D
M3257S	B	B	B	B
M3296H	D	A	D	D
M3432H	A	I	A	A
M3446S	C	B	C	C
M3457E	E	E	E	E
M4386H	B	B	A	A
M4396H	H	H	F	F
M4444H	B	B	A	A
M4UMC19H	A	A	A	A
M4UMC31S	D	A	B	D
M5213S	B	A	B	A
M5295E	C	D	C	C
M5408H	A	A	A	A
M5579S	B	B	B	B
M5UMC95H	A	A	B	B
M6223E	C	C	C	C
M6252H	D	—	D	E
M6280H	E	E	A	A
M6373E	A	E	A	A
M7263E	A	C	A	A
M7391H	C	C	A	A
M7392S	C	C	B	C
M7455H	A	A	C	C
M8110S	C	C	C	C
M8114E	B	B	E	E
M8268H	B	B	B	B
M8585H	A	A	A	A
M8B2369S	B	D	B	D
M8UMC48E	C	C	C	C
M9209E	C	C	A	A
M9266S	A	A	C	C
M9B713S	A	A	B	B
M2UMC34H	D	D	D	—
M6UMC85H	A	A	A	—
M9UMC94H	E	E	B	—
M3UM121X	C	C	C	—
M0UMC130	C	H	—	—

*Probes used to detect RFLPs are from Linkage Genetics, 1515 West 2200 South, Suite C, Salt Lake City, Utah 84119.

Another aspect of this invention is a plant genetic complement characterized by a genetic isozyme typing profile. Isozymes are forms of proteins that are distinguishable, for example, on starch gel electrophoresis, usually by charge and/or molecular weight. The techniques and nomenclature for isozyme analysis are described in, Stuber et al. (1988), which is incorporated by reference.

A standard set of loci can be used as a reference set. Comparative analysis of these loci is used to compare the purity of hybrid seeds, to assess the increased variability in hybrids compared to inbreds, and to determine the identity of seeds, plants, and plant parts. In this respect, an isozyme reference set can be used to develop genotypic "fingerprints."

Table 9 lists the identifying numbers of the alleles at isozyme loci types, and represents the exemplary genetic isozyme typing profile for 87DIA4.

TABLE 9

ISOZYME PROFILE OF 87DIA4

	ISOZYME ALLELE			
LOCUS	87DIA4	2FACC	3AZA1	AQA3
Acph1	2	2	4	4
Adh1	4	4	4	4
Cat3	9	9	9	9
Got1	4	4	4	4
Got2	4	4	4	4
Got3	4	4	4	4
Idh1	4	4	4	4
Idh2	6	6	6	6
Mdh1	6	6	6*	6
Mdh2	3.5	3.5	6	3
Mdh3	16	16	16	16
Mdh4	12	12	12	12
Mdh5	12	12	12	12
Pgm1	9	9	9	9
Pgm2	4	4	4	4
6Pgd1	3.8	3.8	3.8	3.8
6Pgd2	5	5	5	5
Phi1	4	4	4	5

*Allele is probably a 6, but null cannot be ruled out.

The present invention also contemplates a hybrid genetic complement formed by the combination of a haploid genetic complement of the corn plant 87DIA4 with a haploid genetic complement of a second corn plant. Means for combining a haploid genetic complement from the foregoing inbred with another haploid genetic complement can be any method hereinbefore for producing a hybrid plant from 87DIA4. It is also contemplated that a hybrid genetic complement can be prepared using in vitro regeneration of a tissue culture of a hybrid plant of this invention.

A hybrid genetic complement contained in the seed of a hybrid derived from 87DIA4 is a further aspect of this invention. Exemplary hybrid genetic complements are the genetic complements of the hybrid 4033843.

Table 10 shows the identifying numbers of the alleles for the hybrid 4033843, which are exemplary RFLP genetic marker profiles for hybrids derived from the inbred of the present invention. Table 10 concerns 4033843, which has 87DIA4 as one inbred parent.

TABLE 10

RFLP PROFILE FOR 4033843

Probe/Enzyme Combination	Allelic Pair
M0264H	DH
M0306H	AA
M0445E	BC
M1120S	EF
M1234H	AD
M1238H	AE
M1401E	AC
M1406H	AB
M1447H	BB
M1B725E	BB
M2239H	AD
M2297H	AC
M2298E	CC
M2402H	EE
M3212S	AC
M3257S	AB
M3296H	CD
M3432H	AA
M3446S	CF
M3457E	EE
M4386H	BD

TABLE 10-continued

RFLP PROFILE FOR 4033843	
Probe/Enzyme Combination	Allelic Pair
M4396E	HH
M4444H	AB
M4UMC19H	AA
M4UMC31S	AD
M5213S	AB
M5408H	AA
M6223E	BC
M6252H	AD
M6280H	EG
M6373E	AE
M7263E	AA
M7391H	AC
M7392S	AC
M7455H	AB
M8110S	AC
M8114E	BB
M8268H	BL
M8585H	AB
M8B2369S	BB
M8UMC48E	CC
M9209E	AC
M9266S	AA
M9B713S	AA
M2UMC34H	DF
M9UMC94H	EE
M3UM121X	CD
M0UMC130	CC

*Probes used to detect RFLPs are from Linkage Genetics, 1515 West 2200 South, Suite C, Salt Lake City, Utah 84119.

The exemplary hybrid genetic complements of hybrid 4033843 may also be assessed by genetic isozyme typing profiles using a standard set of loci as a reference, set, using, e.g., the same, or a different, set of loci to those described above. Table 11 lists the identifying numbers of the alleles at isozyme loci types and presents the exemplary genetic isozyme typing profile for the hybrid 4033843, which is an exemplary hybrid derived from the inbred of the present invention. Table 11 concerns 4033843, which has 87DIA4 as one inbred parent.

TABLE 11

ISOZYME GENOTYPE FOR HYBRID 4033843	
LOCUS	ISOZYME ALLELES
Acph1	2
Adh1	4
Cat3	9
Got1	4
Got2	4
Got3	4
Idh1	4
Idh2	6
Mdh1	6
Mdh2	3.5
Mdh3	16
Mdh4	12
Mdh5	12
Pgm1	9
Pgm2	4
6-Pgd1	3.8
6-Pgd2	5
Phi1	4

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of the foregoing illustrative embodiments, it will be apparent to those of skill in the art that variations, changes, modifications and alterations may be applied to the composition, methods, and in the steps or in the sequence of steps of the methods described herein, without departing, from the true concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

Armstrong & Green, "Establishment and Maintenance of Friable Embryogenic Maize Callus and the Involvement of L-Proline," *Planta,* 164:207–214, 1985.

Conger et al., "Somatic Embryogenesis from Cultured Leaf Segments of *Zea Mays,*" *Plant Cell Reports,* 6:345–347, 1987.

Duncan et al., "The Production of Callus Capable of Plant Regeneration from Immature Embryos of Numerous *Zea Mays* Genotypes," *Planta,* 165:322–332, 1985.

Fehr (ed.), *Principles of Cultivar Development,* Vol. 1: Theory and Technique, pp. 360–376, 1987.

Gaillard et al., "Optimization of Maize Microspore Isolation and Culture Condition for Reliable Plant Regeneration," *Plant Cell Reports,* 10(2):55, 1991.

Gordon-Kamm et al., "Transformation of Maize Cells and Regeneration of Fertile Transgenic Plants," *The Plant Cell,* 6:603–618, 1990.

Green & Rhodes, "Plant Regeneration in Tissue Cultures of Maize," *Maize for Biological Research,* Plant Molecular Biology Association, pp. 367–372, 1992.

Jensen, "Chromosome Doubling Techniques in Haploids," Haploids and Higher Plants—*Advances and Potentials, Proceedings of the First International Symposium,* University of Guelph, Jun. 10–14, 1974.

Nienhuis et al., "Restriction Fragment Length Polymorphism Analysis of Loci Associated with Insect Resistance in Tomato," *Crop Science,* 27:797–803, 1987.

Pace et al., "Anther Culture of Maize and the Visualization of Embryogenic Microspores by Fluorescent Microscopy," *Theoretical and Applied Genetics,* 73:83 63–869, 1987.

Poehlman & Sleper (eds), *Breeding Field Crops,* 4th Ed., pp. 172–175, 1995.

Rao et al., "Somatic Embryogenesis in Glume Callus Cultures," *Maize Genetics Cooperation Newsletter,* Vol. 60, 1986.

Songstad et al. "Effect of 1-Aminocyclopropate-1-Carboxylic Acid, Silver Nitrate, and Norbornadiene on Plant Regeneration from Maize Callus Cultures," *Plant Cell Reports,* 7:262–265, 1988.

Stuber et al., "Techniques and scoring procedures for starch gel electrophoresis of enzymes of maize *C. Zea mays, L.,*" *Tech. Bull.,* N. Carolina Agric. Res. Serv., Vol. 286, 1988.

Wan et al., "Efficient Production of Doubled Haploid Plants Through Colchicine Treatment of Anther-

Derived Maize Callus," *Theoretical and Applied Genetics*, 77:889–892, 1989.

What is claimed is:

1. Inbred corn seed of the corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

2. The inbred corn seed of claim 1, further defined as an essentially homogeneous population of inbred corn seed designated 87DIA4.

3. The inbred corn seed of claim 1, further defined as essentially free from hybrid seed.

4. An inbred corn plant produced by growing the seed of an inbred corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

5. Pollen of the plant of claim 4.

6. An ovule of the plant of claim 4.

7. An essentially homogeneous population of corn plants produced by growing the seed of an inbred corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

8. A corn plant having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

9. The corn plant of claim 8, further comprising a cytoplasmic factor conferring male sterility.

10. A tissue culture of regenerable cells of inbred corn plaint 87DIA4, wherein the tissue regenerates plants having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

11. The tissue culture of claim 10, wherein the regenerable cells are embryos, meristematic cells, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks, stalks, or protoplasts or callus derived therefrom.

12. A corn plant regenerated from the tissue culture of claim 10, having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

13. An inbred corn plant cell of the corn plant of claim 4 having:

(a) an RFLP genetic marker profile in accordance with the profile shown in Table 8; or

(b) a genetic isozyme typing profile in accordance with the profile shown in Table 9.

14. The inbred corn plant cell of claim 13, having an RFLP genetic marker profile in accordance with the profile shown in Table 8.

15. The inbred corn plant cell of claim 13, having a genetic isozyme typing profile in accordance with the profile shown in Table 9.

16. The inbred corn plant cell of claim 13, having an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 8 and 9.

17. The inbred corn plant cell of claim 13, located within a corn plant or seed.

18. The inbred corn plant of claim 4 having:

(a) an RFLP genetic marker profile in accordance with the profile shown in Table 8; or

(b) a genetic isozyme typing profile in accordance with the profile shown in Table 9.

19. The inbred corn plant of claim 18, having an RFLP genetic marker profile in accordance with the profile shown in Table 8.

20. The inbred corn plant of claim 18, having a genetic isozyme typing profile in accordance with the profile shown in Table 9.

21. The inbred corn plant of claim 18, having an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 3 and 9.

22. A process of preparing corn seed, comprising crossing a first parent corn plant with a second parent corn plant, wherein said first or second corn plant is the inbred corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192, wherein seed is allowed to form.

23. The process of claim 22, further defined as a process of preparing hybrid corn seed, comprising crossing a first inbred corn plant with a second, distinct inbred corn plant, wherein said first or second corn plant is the inbred corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

24. The process of claim 23, wherein crossing comprises the steps of:

(a) planting in pollinating proximity seeds of said first and second inbred corn plants;

(b) cultivating the seeds of said first and second inbred corn plants into plants that bear flowers;

(c) emasculating the male flowers of said first or second inbred corn plant to produce an emasculated corn plant;

(d) allowing cross-pollination to occur between said first and second inbred corn plants; and

(e) harvesting seeds produced on said emasculated corn plant.

25. The process of claim 24, further comprising growing said harvested seed to produce a hybrid corn plant.

26. Hybrid corn seed produced by the process of claim 23.

27. A hybrid corn plant produced by the process of claim 25.

28. The hybrid corn plant of claim 27, wherein the plant is a first generation (F_1) hybrid corn plant.

29. The corn plant of claim 8, further comprising a single gene conversion.

30. The corn plant of claim 29, wherein the single gene was stably inserted into a corn genome by transformation.

31. The single gene conversion of the corn plant of claim 29, where the gene is a dominant allele.

32. The single gene conversion of the corn plant of claim 29, where the gene is a recessive allele.

33. The single gene conversion corn plant of claim 29, where the gene confers herbicide resistance.

34. The single gene conversion of the corn plant of claim 29, where the gene confers insect resistance.

35. The single gene conversion of the corn plant of claim 29, where the gene confers resistance to bacterial, fungal, or viral disease.

36. The single gene conversion of the corn plant of claim 29, wherein the gene confers male sterility.

37. The single gene conversion of the corn plant of claim 29, where the gene confers waxy starch.

38. The single gene conversion of the corn plant of claim 29, where the gene confers improved nutritional quality.

39. The single gene conversion of the corn plant of claim 29, where the gene confers enhanced yield stability.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145 Page 1 of 6

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 5, at lines 46-47, please delete "Ear-Fresh Husk The color of the husks 1 to 2 weeks after pollination scored as Color: green, red, or purple" and substitute therefor --Ear-Fresh Husk Color: The color of the husks 1 to 2 weeks after pollination scored as green, red, or purple--.

In col. 5, at lines 56-57, please delete "Ear-Number Per The average number of ears per plant Stalk:" and substitute therefor --Ear-Number Per Stalk: The average number of ears per plant--.

In col. 5, at lines 58-59, please delete "Ear-Shank The average number of internodes on the ear shank. Internodes:" and substitute therefor --Ear-Shank Internodes: The average number of internodes on the ear shank--.

In col. 6, at lines 51-53, please delete "Kernel-Aleurone The color of the aleurone scored as white, pink, tan, brown, Color: bronze, red, purple, pale purple, colorless, or variegated" and substitute therefor --Kernel-Aleurone Color: The color of the aleurone scored as white, pink, tan, brown, bronze, red, purple, pale purple, colorless, or variegated--.

In col. 6, at lines 57-58, please delete "Kernel-Endosperm The color of the endosperm scored as white, pale yellow, or Color: yellow" and substitute therefor --Kernel-Endosperm Color: The color of the endosperm scored as white, pale yellow, or yellow--.

In col. 6, at lines 59-60, please delete "Kernel-Endosperm The type of endosperm scored as normal, waxy, or opaque. Type:" and substitute therefor --Kernel-Endosperm Type: The type of endosperm scored as normal, waxy, or opaque--.

CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 6, at lines 65-66, please delete "Kernel-Number Per The average number of kernels in a single row. Row:" and substitute therefor --Kernel-Number Per Row: The average number of kernels in a single row--.

In col. 7, at lines 1-3, please delete "Kernel-Pericarp The color of the pericarp scored as colorless, red-white crown, tan, Color: bronze, brown, light red, cherry red, or variegated" and substitute therefor -- Kernel-Pericarp Color: The color of the pericarp scored as colorless, red-white crown, tan, bronze, brown, light red, cherry red, or variegated--.

In col. 7, at lines 4-6, please delete "Kernel-Row The direction of the kernel rows on the ear scored as straight, Direction: slightly curved, spiral, or indistinct (scattered)" and substitute therefor --Kernel-Row Direction: The direction of the kernel rows on the ear scored as straight, slightly curved, spiral, or indistinct (scattered)--.

In col. 7, at lines 30-33, please delete "Leaf-Longitudinal A rating of the number of longitudinal creases on the leaf surface 1 Creases: to 2 weeks after pollination. Creases are scored as absent, few, or many" and substitute therefor --Leaf-Longitudinal Creases: A rating of the number of longitudinal creases on the leaf surface 1 to 2 weeks after pollination. Creases are scored as absent, few, or many--.

In col. 7, at lines 34-36, please delete "Leaf-Marginal A rating of the waviness of the leaf margin 1 to 2 weeks after Waves: pollination. Rated as none, few, or many" and substitute therefor --Leaf-Marginal Waves: A rating of the waviness of the leaf margin 1 to 2 weeks after pollination. Rated as none, few, or many--.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 7, at lines 40-43, please delete "Leaf-Sheath A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks Anthocyanin: after pollination, scored as absent, basal-weak, basal-strong, weak or strong" and substitute therefor --Leaf-Sheath Anthocyanin: A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks after pollination, scored as absent, basal-weak, basal-strong, weak or strong--.

In col. 7, at lines 44-46, please delete "Leaf-Sheath A rating of the pubescence of the leaf sheath. Ratings are taken 1 Pubescence: to 2 weeks after pollination and scored as light, medium, or heavy" and substitute therefor --Leaf-Sheath Pubescence: A rating of the pubescence of the leaf sheath. Ratings are taken 1 to 2 weeks after pollination and scored as light, medium, or heavy--.

In col. 8, at lines 19-21, please delete "Stalk-Brace Root The color of the brace roots observed 1 to 2 weeks after pollination Color: as green, red, or purple" and substitute therefor --Stalk-Brace Root Color: The color of the brace roots observed 1 to 2 weeks after pollination as green, red, or purple--.

In col. 8, at lines 27-29, please delete "Stalk-Internode The direction of the stalk internode observed after pollination as Direction: straight or zigzag" and substitute therefor --Stalk-Internode Direction: The direction of the stalk internode observed after pollination as straight or zigzag--.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 8, at lines 30-31, please delete "Stalk-Internode The average length of the internode above the primary ear. Length:" and substitute therefor --Stalk Internode Length: The average length of the internode above the primary ear--.

In col. 8, at lines 35-36, please delete "Stalk-Internode With The average number of nodes having brace roots per plant. Brace Roots:" and substitute therefor --Stalk-Internode With Brace Roots: The average number of nodes having brace roots per plant--.

In col. 8, at lines 65-66, please delete "Tassel-Branch The average number of primary tassel branches. Number:" and substitute therefor --Tassel-Branch Number: The average number of primary tassel branches--.

In col. 9, at lines 7-9, please delete "Tassel-Peduncle The average length of the tassel peduncle, measured from the base of the flag leaf to the base Length: of the bottom tassel branch" and substitute therefor --Tassel-Peduncle Length: The average length of the tassel peduncle, measured from the base of the flag leaf to the base of the bottom tassel branch--.

In col. 13, at line 26, delete "3AZA1" and substitute therefor --AQA3--.

In col. 14, at line 52, delete "87DIA114" and substitute therefor --87DIA4--.

In col. 30, at line 4, , delete "DEKALB Plant Genetics" and substitute therefor --DEKALB Genetics Corporation--.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At col. 17, in Table 5 delete all rows under "4. EAR" and ending with "Endosperm Color" and substitute therefor the following rows --

4. EAR				
Silk Color	Pink	Pink	—	Red
Number Per Stalk	1.0	1.0	1.0	1.4
Position (attitude)	Upright	—	—	Upright
Length cm.	14.6	16.0	14.6	15.6
Shape	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Diameter cm.	4.0	3.8	4.0	3.9
Weight gm.	104.9	100.6	103.2	107.6
Shank Length cm.	10.3	14.1	10.1	9.6
Husk Bract	Short	Short	Short	Short
Husk Cover cm.	6.4	2.5	4.4	3.7
Husk Color Fresh	Green	Green	Green	Green
Husk Color Dry	Buff	Buff	Buff	Buff
Cob Diameter cm.	2.3	2.4	2.3	2.1
Cob Color	Red	—	Red	Red
Shelling Percent	87.7	80.6	89.0	83.3

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

5. KERNEL

Row Number	14.6	14.8	16.3	15.3
Number Per Row	32.1	27.1	29.3	29.7
Row Direction	Curved	Curved	Curved	Curved
Type	Dent	—	Dent	—
Cap Color	Yellow	Yellow	Yellow	Yellow
Side Color	Deep Yellow	—	Orange	
Length (depth) mm.	11.1	9.4	10.9	10.3
Width mm.	7.8	8.0	7.4	7.8
Thickness	3.9	5.2	4.4	4.2
Weight of 1000K gm.	269.0	252.4	233.0	247.8
Endosperm Type	Normal	Normal	Normal	Normal
Endosperm Color	Yellow	Yellow	Yellow	Yellow–.

Signed and Sealed this

Twenty-seventh Day of February, 2001

Attest:

Nicholas P. Godici

NICHOLAS P. GODICI

Attesting Officer

Acting Director of the United States Patent and Trademark Office

EXHIBIT E

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Date(s) Docketed: Status Check - Decision on Appeal rcvd. Response from Examiner?
6/30/05
APR 04 2005
Client: DEKA:276US
Attorney(s): DLP, REH
Initials:

Ex parte Francis L. Garing

Appeal No. 2004-2343[1]
Application No. 09/772,520

MAILED
MAR 31 2005
U.S. PATENT AND TRADEMARK OFFICE
BOARD OF PATENT APPEALS
AND INTERFERENCES

ON BRIEF[2]

Before SCHEINER, ADAMS and GREEN, Administrative Patent Judges.

ADAMS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on the appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 3, 6, 11, 14-20 and 24-31. The examiner has indicated that claims 1, 2, 5, 7-10, 12, 13 and 21-23 are allowable. Claim 4 is cancelled.

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[1] This appeal is substantially similar to Appeal No. 2004-1503, Application No. 09/606,808; Appeal No. 2004-1506, Application No. 09/788,334; Appeal No. 2004-1968, Application No. 10/00,0311; Appeal No. 2004-2317, Application No. 09/771,938; and Appeal No. 2005-0396, Application No. 10/077,589, which all share the same assignee, Monsanto Company, the parent of wholly-owned subsidiary DeKalb Genetics Corporation. Accordingly we have considered these appeals together.

[2] We note that appellant waived his request for Oral Hearing. See Paper received December 3, 2004.

Claims 3, 6, 15, 16, 17, 27, 28 and 30 are illustrative of the subject matter on appeal and are reproduced below. In addition, for convenience, we have reproduced allowable claims 2 and 5 below:

2. A population of seed of the corn variety I026458, wherein a sample of the seed of the corn variety I026458 was deposited under ATCC Accession No. PTA-3228.

3. The population of seed of claim 2, further defined as an essentially homogeneous population of seed.

5. A corn plant produced by growing a seed of the corn variety I026458, wherein a sample of the seed of the corn variety I026458 was deposited under ATCC Accession No. PTA-3228.

6. The corn plant of claim 5, having:
   (a) an SSR profile in accordance with the profile shown in Table 6; or
   (b) an isozyme typing profile in accordance with the profile shown in Table 7.

15. A corn plant capable of expressing all the physiological and morphological characteristics of the corn variety I026458, wherein a sample of the seed of the corn variety I026458 was deposited under ATCC Accession No. PTA-3228.

16. The corn plant of claim 15, further comprising a nuclear or cytoplasmic gene conferring male sterility.

17. A tissue culture of regenerable cells of a plant of corn variety I026458, wherein the tissue is capable of regenerating plants capable of expressing all the physiological and morphological characteristics of the corn variety I026458, wherein a sample of the seed of the corn variety I026458 was deposited under ATCC Accession No. PTA-3228.

27. The corn plant of claim 5, further defined as having a genome comprising a single locus conversion.

28. The corn plant of claim 27, wherein the single locus was stably inserted into a corn genome by transformation.

30. The corn plant of claim 27, wherein the locus confers a trait selected from the group consisting of herbicide tolerance; insect resistance; resistance to bacterial, fungal, nematode or viral disease; yield enhancement; waxy

starch; improved nutritional quality; enhanced yield stability; male sterility and restoration of male fertility.

The references relied upon by the examiner are:

Hunsperger et al. (Hunsperger)	5,523,520	Jun. 4, 1996

Eshed et al. (Eshed), "Less-Than-Additive Epistatic Interactions of Quantitative Trait Loci in Tomato," Genetics, Vol. 143, pp. 1807-17 (1996)

Kraft et al. (Kraft), "Linkage Disequilibrium and Fingerprinting in Sugar Beet," Theoretical and Applied Genetics, Vol. 101, pp. 323-36 (2000)

GROUNDS OF REJECTION

Claim 3 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "an essentially homogeneous population of seed."

Claim 14 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "[a]n essentially homogeneous population of corn plants produced by growing the seed of the corn variety I026458."

Claims 6 and 11 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "in accordance with."

Claims 15, and 17-20[3] stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "capable of expressing."

[3] According to the examiner (Answer, pages 12 and 13), since claims 18 and 19 depend from claim 17 they are included in this rejection.

Claims 16 and 27-30[4] stand rejected under 35 U.S.C. § 112, second paragraph as failing to limit the scope of the claims from which they depend.

Claim 28 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of "the article 'a' in the recitation 'wherein the single locus was stably inserted into a corn genome.'"

Claim 30 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrases "yield enhancement," "improved nutritional quality," and "enhanced yield stability."

Claims 6, 11, 24-31[5] stand rejected under the written description provision of 35 U.S.C. § 112, first paragraph.

Claims 27-30 stand rejected under the enablement provision of 35 U.S.C. § 112, first paragraph.

We reverse.

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[4] According to the examiner (Answer, page 4), "[c]laims ... 27-30 ... stand rejected under 35 U.S.C. [§] 112, second paragraph..." The examiner, however, provides no explanation as to why claim 29 is rejected. We can only assume that since claim 29, as well as claims 28 and 30, each depend from claim 27, they are rejected for the same reason as claim 27. Accordingly, we have included claims 28-30 with this ground of rejection.

[5] We recognize the examiner's statement (Answer, page 3), "[c]laim 26 was objected to in the Office Action mailed 23 September 2003, as being in improper dependent form for failing to further limit the subject matter of previous claim. Appellant did not address this objection. An objection to a claim, however, is the subject matter of a petition, and is not properly before us on appeal. Nevertheless, we make the following observation regarding claim 26, and encourage the examiner and appellant to work together to remedy this issue, prior to any further action on the merits.

According to appellant's specification (page 20), a F_1 hybrid is "[t]he first generation progeny of the cross of two plants." Therefore, as we understand the prosecution history as well as the language of the claims, claims 24 and 25 to refer to F_1 hybrids. In this regard, we note that similar claims, directed to a different corn variety, were presented for our review in Appeal Nos. 2004-1506 and 2004-2317. During the oral hearing in Appeal Nos. 2004-1506 and 2004-2317, appellant's representative confirmed that all claims drawn to hybrid plants or hybrid seeds (see e.g., claims 24 and 25) refer to F_1 hybrids. Accordingly, it appears that claim 26 fails to further limit claim 25 from which it depends.

BACKGROUND

The present "invention relates to inbred corn seed and plants of the variety designated I026458, and derivatives and tissue cultures thereof." Specification, page 2. According to appellant (specification, page 28), "[a] description of the physiological and morphological characteristics of corn plant I026458 is presented in Table 3" of the specification, pages 28-29. On this record the examiner has indicated that claims drawn to plants, plant parts, and seed of the corn variety designated I026458 are allowable. See e.g., claims 1, 2, 5, 7-10, 12 and 13, and Answer, page 2, wherein the examiner states "[c]laims 1, 2, 5, 7-10, 12 [and] 13 ... are allowed."

A second aspect of the present invention comprises hybrid plants and processes "for producing [first generation (F_1) hybrid[6]] corn seeds or plants, which ... generally comprise crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is a plant of the variety designated I026458." Specification, pages 7-9. On this record the examiner has indicated that claims drawn to a process of producing corn seed wherein the process comprises crossing a first parent corn plant with a second parent corn plant are allowable. See e.g., claims 21-23 and Answer, page 2, wherein the examiner states claims "21-23 are allowed."

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[6] According to the specification (page 21), a F_1 hybrid is "[t]he first generation progeny of the cross of two plants." During oral hearing, appellant confirmed that all claims drawn to hybrid plants or hybrid seeds (see e.g., claims 24 and 25) refer to F_1 hybrids.

A third aspect of the present invention comprises single locus converted plants of the corn variety I026458. Specification, page 6. As appellant explains (specification, page 23, emphasis added), single locus converted (conversion) plants are those plants

> which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. A single locus may comprise one gene, or in the case of transgenic plants, one or more transgenes integrated into the host genome at a single site (locus).

As appellant explains (specification, page 31):

> Many single locus traits have been identified that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques. Single locus traits may or may not be transgenic; examples of these traits include, but are not limited to, male sterility, waxy starch, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, male fertility, enhanced nutritional quality, industrial usage, yield stability, and yield enhancement. These genes are generally inherited through the nucleus, but may be inherited through the cytoplasm. Some known exceptions to this are genes for male sterility, some of which are inherited cytoplasmically, but still act as single locus traits.

A final aspect of the present invention is directed to a process of producing an inbred corn plant derived from a plant of the corn variety I026458. See e.g., claim 31. According to appellant's specification (bridging paragraph, pages 10-11),

> the present invention provides a method of producing an inbred corn plant derived from the corn variety I026458, the method comprising the steps of: (a) preparing a progeny plant derived from corn variety I026458, wherein said preparing comprises crossing a plant of the corn variety I026458 with a second corn plant, and

> wherein a sample of the seed of corn variety I026458 has been deposited under ATCC Accession No. PTA-3228; (b) crossing the progeny plant with itself or a second plant to produce a seed of a progeny plant of a subsequent generation; (c) growing a progeny plant of a subsequent generation from said seed of a progeny plant of a subsequent generation and crossing the progeny plant of a subsequent generation with itself or a second plant; and (d) repeating steps (c) and (d) for an addition 3-10 generations to produce an inbred corn plant derived from the corn variety I026458. In the method, it may be desirable to select particular plants resulting from step (c) for continued crossing according to steps (b) and (c). By selecting plants having one or more desirable traits, an inbred corn plant derived from the corn variety I026458 is obtained which possesses some of the desirable traits of corn variety I026458 as well potentially other selected traits.

According to the examiner (Answer, page 36), "[t]he patentability of the method of claim 31 does not lie in the acts of the process, which are the simple acts of crossing corn plants, allowing progeny seed to be produced, and growing progeny plants from the seed...." Therefore, as we understand this aspect of the claimed invention (e.g., claim 31), the intent is not to claim a specific inbred corn plant resulting from the claimed process. See claim 31. Instead, as we understand it, claim 31 is drawn to a process wherein an inbred corn plant is derived from the corn variety I026458.

As appellant explains (specification, page 3),

> The development of uniform corn plant hybrids requires the development of homozygous inbred plants, the crossing of these inbred plants, and the evaluation of the crosses. Pedigree breeding and recurrent selection are examples of breeding methods used to develop inbred plants from breeding populations. Those breeding methods combine the genetic backgrounds from two or more inbred plants or various other broad-based sources into breeding pools from which new inbred plants are developed by selfing and selection of desired phenotypes. The new inbreds are crossed with other inbred plants and the hybrids from these crosses are evaluated to determine which of those have commercial potential.

We emphasize, that while "new inbreds" having commercial potential may result from the method set forth in claim 31, the claim does not encompass any specific plant that is produced as a result of the method. Rather the claim encompasses only a method of producing an inbred corn plant that is "derived" from the corn variety I026458. The examiner has indicated that a claim drawn to a corn plant of the corn variety I026458 is allowable. See e.g., claim 5, and Answer, page 2, wherein the examiner states that claim 5 is allowed.

Against this backdrop, we now consider the rejections of record.

DISCUSSION

Definiteness:

Claims 3, 6, 11, 14-20 and 27-30 stand rejected under 35 U.S.C. § 112, second paragraph. For the following reasons we reverse.

Claim 3

Claim 3 depends from independent claim 2, and stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "an essentially homogeneous population of seed...." Answer, page 4. According to the examiner (id.), claim 2 is drawn to "'[a] population of seed of the corn variety I026458, wherein a sample of the seed of the corn variety I026458 was deposited under ATCC Accession No. PTA-3228." Thus, the examiner finds (Answer, page 5), the population of seed set forth in claim 2 "is a homogeneous population of seed of corn variety I026458." Accordingly, the examiner finds

(id.), "[t]he recitation, 'essentially homogeneous,' in claim 3 ... appear[s] to be superfluous."

However, as disclosed in appellant's specification (page 5),

> [e]ssentially homogeneous populations of inbred seed are those that consist essentially of the particular inbred seed, and are generally free from substantial numbers of other seed, so that the inbred seed forms between about 90% and about 100% of the total seed, and preferably, between about 95% and about 100% of the total seed.

Accordingly, we disagree with the examiner's assertion (Answer, page 6) that claim 3 is unclear simply because it may contain seed other than the seed of the corn variety I026458. We remind the examiner that claim language must be analyzed "not in a vacuum, but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary skill in the pertinent art." In re Moore, 439 F.2d 1232, 1235, 169 USPQ 236, 238 (CCPA 1971). Here, notwithstanding appellant's comments[7], it is our opinion that a person of ordinary skill in the art would recognize that an essentially homogeneous population of seed of the corn variety I026458 is a population of seed that is generally free from substantial numbers of other seed, e.g., wherein corn variety I026458 seed forms between about 90% and about 100% of the total seed in the population.[8]

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[7] According to appellant (Brief, page 7), an essentially homogeneous population of seed, is a population of seed that could be of non-uniform size and shape.

[8] Cf. the examiner's statement (Answer, page 6), "amending claim 3 to read '[a]n essentially homogeneous population of corn seeds consisting essentially of seed of claim 1', would obviate this rejection."

Accordingly, we reverse the rejection of claim 3 under 35 U.S.C. § 112, second paragraph.

Claim 14

Claim 14 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "[a]n essentially homogeneous population of corn plants produced by growing the seed of the corn variety I026458." Answer, page 6. According to the examiner (id.), "[t]he I026458 seed can only produce I026458 plants. ... [Therefore,] [t]he population can ... only consist of I026458 plants." Accordingly, the examiner finds it unclear "why the population is referred to as 'essentially homogeneous,' since such populations can comprise more than one variety of plant." Answer, bridging sentence, pages 6-7.

As appellant discloses (specification, page 6), "[t]he population of inbred corn seed of the invention can further be particularly defined as being essentially free from hybrid seed. The inbred seed population may be separately grown to provide an essentially homogeneous population of inbred corn plants designated I026458." As we understand the claim, growing the seed of claim 3, for example, would produce an essentially homogeneous population of corn plants of the corn variety I026458.[9]

[9] Cf. The examiner's statement (Answer, page 8), amending claim 14 "to read, '[a]n essentially homogeneous population of corn plants produced by growing a population of corn seed consisting essentially of the seed of corn plant I026458...' would obviate the rejection."

In addition, we direct the examiner's attention to Appeal No. 2005-0396, wherein a claim similar to claim 14 was presented for our review. In Appeal No. 2005-0396, the examiner of record indicated that claim 14, directed to "[a]n essentially homogeneous population of corn plants produced by growing the seed of the corn variety I180580...." was allowable. Accordingly, we find that the examiner has treated claim 14 in a manner that is inconsistent with the prosecution of claim 14 in 2005-0396. As we understand it, the only difference between claim 14 as it appears in Appeal No. 2005-0396 and the instant appeal is the variety of corn seed from which the plant is produced.

Accordingly we reverse the rejection of claim 14 under 35 U.S.C. § 112, second paragraph.

Claims 6 and 11

Claims 6 and 11 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "in accordance with." According to the examiner (Answer, page 9), it is unclear if a plant "that generally follows the trend of the profile of Table 6, but which differs at one or a few loci, [would] be considered in 'conformity' or 'in accordance' with the profile of Table 6."

On this record, we understand the phrase "in accordance with" as it is used in claims 6 and 11 to mean "the same"[10] Stated differently, we understand the claims to read:

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[10] Cf. Appeal Nos. 2004-1506 and 2004-2317, which use similar language for claims directed to different corn varieties. In this regard, we note that during the February 10, 2005 oral hearing in Appeal Nos. 2004-1506 and 2004-2317, appellant's representative confirmed that the phrase "in accordance with" was intended to mean "the same".

6. The corn plant of claim 5, having:
   (a) the same SSR profile as shown in Table 6; or
   (b) the same isozyme typing profile as shown in Table 7.

11. The plant part of claim 10, wherein said cell is further defined as having:
   (a) The same SSR profile as shown in Table 6; or
   (b) The same isozyme typing profile as shown in Table 7.

Accordingly we reverse the rejection of claims 6 and 11 under 35 U.S.C. § 112, second paragraph.

Claims 15 and 17-20

Claims 15, and 17-20 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "capable of expressing," or "capable of regenerating." According to the examiner (Answer, page 9), the claims do "not make clear if the plant actually expresses the traits, or when or under what conditions the traits are expressed." In this regard, the examiner finds (Answer, page 10),

> while the plant has the capacity to express the characteristics, for some reason it may not. Certain characteristics of a plant are expressed only at certain times of its life cycle, and are incapable of being expressed at other times. The colors of flower parts such as silks, or fruit parts such as husks, are examples. The promoters of many genes conferring traits require a transcription factor to become active. Is a plant that has such a gene, but not the transcription factor, considered "capable of expressing" that gene, and the trait associated with that gene, and is such a plant encompassed by the claims?

To address the examiner's concerns, we find it sufficient to state that if a plant has the capacity to express the claimed characteristics it meets the requirement of the claim regarding "capable of," notwithstanding that due to a particular phase of the life cycle the plant is not currently expressing a particular

characteristic. Alternatively, if a plant is incapable of expressing the claimed characteristics at any phase of the life cycle, because it lacks, for example, the "transcription factor" required for expression – such a plant would not meet the requirement of the claim regarding "capable of."

Here, we find the examiner's extremely technical criticism to be a departure from the legally correct standard of considering the claimed invention from the perspective of one possessing ordinary skill in the art.[11] In our opinion, a person of ordinary skill in the art would understand what is claimed. Amgen Inc. v. Chugai Pharmaceutical Co., Ltd., 927 F.2d 1200, 1217, 18 USPQ2d 1016, 1030 (Fed. Cir. 1991). We find the same to be true for the phrase "capable of" as set forth in claims 17-20.

Accordingly we reverse the rejection of claims 15, and 17-20 under 35 U.S.C. § 112, second paragraph.

Claims 16 and 27-30

Claims 16 and 27-30 stand rejected under 35 U.S.C. § 112, second paragraph as failing to limit the scope of the claims from which they depend. According to the examiner (Answer, page 11), since the plant set forth in claim 16 is male sterile it cannot express all the morphological and physiological characteristics of the male fertile corn variety I026458. Similarly, the examiner finds it unclear whether the plant set forth in claim 27 has all the characteristics of the plant set forth in claim 5, from which claim 27 depends. Id. In response,

[11] Cf. Digital Equipment Corp. v. Diamond, 653 F.2d 701, 724, 210 USPQ 521, 546 (CA 1981).

appellant asserts (Brief, pages 9-10), claims 16 and 27 simply add a further limitation to the claims from which they depend. We agree.

For example, claim 16 reads on a corn plant capable of expressing all the physiological and morphological characteristics of the corn variety I026458, further comprising a nuclear or cytoplasmic gene conferring male sterility. In our opinion, the claims reasonably apprise those of skill in the art of their scope. Amgen, As set forth in Shatterproof Glass Corp. v. Libbey-Owens Ford Co., 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir. 1985), "[i]f the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more."

Accordingly we reverse the rejection of claims 16 and 27-30 under 35 U.S.C. § 112, second paragraph.

Claim 28

Claim 28 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of "the article 'a' in the recitation 'wherein the single locus was stably inserted into a corn genome.'" According to the examiner (Answer, page 13), "[t]he recitation does not make clear if the genome is that of I026458 or that of a different corn plant."

According to appellant's specification (page 23, emphasis removed), a "Single Locus Converted (Conversion) Plant" refers to

> [p]lants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in

> addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. A single locus may comprise one gene, or in the case of transgenic plants, one or more transgenes integrated into the host genome at a single site (locus).

Accordingly, we agree with appellant (Brief, page 12) "[t]he single locus referred to in claim 28 may or may not have been directly inserted into the genome of the claimed plant." As we understand the claim, and arguments of record, claim 28 presents two possibilities: (1) the single locus is directly inserted into the claimed plant and nothing further need be done; or (2) the single locus is directly inserted into a different plant, which is then used to transfer the single locus to the claimed plant through use of the plant breeding technique known as backcrossing.

In our opinion, the claim reasonably apprises those of skill in the art of its scope. Amgen. Accordingly, we reverse the rejection of claim 28 under 35 U.S.C. § 112, second paragraph.

Claim 30

Claim 30 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrases "yield enhancement," "improved nutritional quality," and "enhanced yield stability." According to the examiner the terms "yield enhancement," "improved nutritional quality," and "enhanced yield stability" are relative and have no definite meaning. Answer, page 14. The examiner is correct (Answer, page 14), when a word of degree is used appellant's specification must provide some standard for measuring that degree.

Seattle Box. Co. v. Industrial Crating & Packing, Inc., 731 F.2d 818, 826, 221 USPQ 568, 573-574 (Fed. Cir. 1984).

On this record, appellant asserts (Brief, page 12), it is "understood the enhancement of yield or yield stability and improved nutritional quality is relative to a plant lacking the single locus. The metes and bounds of the claim are thus fully understood by one of skill in the art and the use of the terms is not indefinite." On reflection, we agree with appellant. The fact that some claim language is not mathematically precise does not per se render the claim indefinite. Seattle Box. As set forth in Shatterproof Glass, "[i]f the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more." In our opinion, a person of ordinary skill in the art would have understood the enhancement of yield or yield stability and improved nutritional quality is relative to a plant lacking the single locus.

Accordingly we reverse the rejection of claim 30 under 35 U.S.C. § 112, second paragraph.

Written Description:

Claims 6, 11, 24, 25 and 27-31 stand rejected under 35 U.S.C. § 112, first paragraph, as the specification fails to adequately describe the claimed invention. For the following reasons, we reverse.

Claims 24-26[12]

Claims 24-26 ultimately depend from claim 23. On this record, the examiner has indicated that claim 23 is allowable. Answer, page 2. The examiner finds (Answer, page 16), claims 24-26 are drawn to a hybrid plant or seed "produced by crossing inbred corn plant I026458 with any second, distinct inbred corn plant."

As we understand it, based on this construction of claims 24-26, the examiner is of the opinion that since the hybrids inherit only ½ of their diploid[13] set of chromosomes from the plant of corn variety I026458, a person of skill in the art would not have viewed the teachings of the specification as sufficient to demonstrate that appellant was in possession of the genus of hybrid seeds and plants encompassed by claims 24-26. According to the examiner (Answer, page 22), "[t]he fact that any hybrid plant will inherit half of its alleles from I026458 then does not provide sufficient description of the morphological and physiological characteristics expressed by the claimed hybrid plants."

There is no doubt that the expressed gene products of a hybrid plant, e.g., the morphological and physiological traits, of I026458 and a non-I026458 corn plant will depend on the combination of the genetic material inherited from both parents. See Answer, page 23. Nevertheless, we disagree with the examiner's

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[12] We recognize, as does the examiner (Answer, page 22) that appellant's reference to claims 22-26 (Brief, page 14) was intended to be a reference to claims 24 and 25.

[13] According to appellant's specification (page 21), diploid means "a cell or organism having two sets of chromosomes."

conclusion (id.) that "[t]he fact that any hybrid plant will inherit half of its alleles from I026458 then does not provide sufficient description of the morphological and physiological characteristics expressed by the claimed hybrid plants."

On these facts, we find it necessary to take a step back and consider what is claimed. The claims are drawn to a F_1 hybrid seed (claim 24) or plant (claim 25) resulting from a cross between a plant of corn variety I026458 and a non-I026458 corn variety. The claims do not require the hybrid to express any particular morphological or physiological characteristic. Nor do the claims require that a particular non-I026458 corn variety be used.[14] All that is required by the claims is that the hybrid has one parent that is a plant of corn variety I026458. Since the examiner has indicated that the seed and the plant of the corn variety I026458 are allowable (see claims 1 and 5), there can be no doubt that the specification provides an adequate written description of this corn variety. In addition, the examiner appears to recognize (Answer, pages 24-25) that appellant's specification describes an exemplary hybrid wherein one parent was a plant of the corn variety I026458, see e.g., specification, pages 53-57. Accordingly, it is unclear to this merits panel what additional description is necessary.

As set forth in Reiffin v. Microsoft Corp., 214 F.3d 1342, 1345, 54 USPQ2d 1915, 1917 (Fed. Cir. 2000), the purpose of the written description

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[14] According to appellant (Brief, page 16), "hundreds or even thousands of different inbred corn lines were well known to those of skill in the art prior to the filing [date] of the instant application, each of which could be crossed to make a hybrid plant with in the scope of the claims."

requirement is to "ensure that the scope of the right to exclude, as set forth in the claims does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification." Here the hybrid seed or plant has one parent that is a plant of the corn variety I026458. To that end, to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). For the foregoing reasons it is our opinion that appellant has provided an adequate written description of the subject matter set forth in claims 24-26.[15]

We recognize the examiner's argument relating to SSR and isozyme markers (Answer, pages 28-33), as well as the examiner's arguments concerning a correlation between the hybrid's genome structure and the function of the hybrid plant (Answer, pages 24-27). However, for the foregoing reasons, we are not persuaded by these arguments.

Claims 6 and 11

Claims 6 and 11 depend ultimately upon claim 5. On this record, the examiner has indicated that claim 5 is allowable. Answer, page 2.

According to the examiner (Answer, page 17), while the specification provides the locus names and allele numbers of the SSR markers, the specification does not provide the actual nucleotide sequences that make up the

[15] Again, we note as set forth in n. 3. that claim 26 does not appear to further limit the scope of claim 25 from which it depends.

markers. According to the examiner (id.), "names of loci alone do not describe the structures of the markers themselves. Without a description of the sequences of the markers, one cannot confirm their presence." In response, appellant points out (Brief, page 13), "the SSR markers were from Celera AgGen, Inc., which provides a commercial service for genotyping of maize varieties." In other words, a person of ordinary skill in the art could use the commercially available service provided by Celera AgGen, Inc. to determine whether a corn plant produced by growing a seed of the corn variety I026458 has an SSR profile which is the same as that shown in Table 6. Therefore, it is unclear to this panel why the examiner believes that such a disclosure fails to provide adequate written descriptive support for the claimed invention.[16] Accordingly, we are not persuaded by the examiner's argument.

Regarding the isozyme typing profile, the examiner finds (Answer, page 17), "Table 7 provides names of loci where isozyme markers reside, for three different corn plants, and a numerical value that represents the numbers of alleles at isozyme loci types. The nucleotide sequences that make up these loci are not described." In response, appellant points out (Brief, page 13), the isozyme "markers are well known and isozyme analysis in general [is] very well known having been used for decades." In this regard, we remind the examiner

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[16] We are not persuaded by the examiner's assertion (Answer, page 31) "that the [commercially available] service used to detect SSR markers is currently available is not a guarantee that it will remain so for the life of a patent issuing from the application." Cf. In re Metcalfe, 410 F.2d 1378, 1382, 161 USPQ 789, 792-3 (CCPA 1969).

that the inquiry into whether the description requirement is met must be determined on a case-by-case basis and is a question of fact. In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). A description as filed is presumed to be adequate; unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. See e.g., In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. Accordingly, it is the examiner who has the initial burden of establishing by a preponderance of evidence that a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. Wertheim, 541 F.2d at 263, 191 USPQ at 97. On this record, the examiner provides no evidence to support the assertion that simply because appellant has not provided the sequences that make up the loci for particular isozymes, appellant's specification does not adequately describe the claimed invention. Accordingly, we are not persuaded by the examiner's argument.

The examiner finds (Answer, page 21), claims 6 and 11 require that the claimed plant or plant cell exhibit either the claimed SSR profile or the isozyme profile. According to the examiner (id.), "[t]he genome of the cells of the I026458 seed deposited with the ATCC has both the SSR profile and the isozyme typing profile shown in Tables 6 and 7 for that plant. No plant is described in the specification that has one genetic marker profile but not the other." The examiner's concern appears to be misplaced. To the extent that the examiner is

concerned that the claim is open to read on a plant other than a corn plant produced by growing a seed of the corn variety I026458, we remind the examiner that both claims 6 and 11 ultimately depend from claim 5[17], which is drawn to "[a] corn plant produced by growing a seed of the corn variety I026458...."

It appears that the examiner may have read claims 6 and 11 as drawn to a corn plant or plant cell having only one of the recited profiles. However, as we understand claims 6 and 11, determining whether the claimed corn plant (claim 6) or plant cell (claim 11) has one of the profiles does not mean that the plant, or plant cell would not also exhibit the other profile.

In addition, we direct the examiner's attention to claims 6 and 11 of Appeal No. 2005-0396. As we understand it, notwithstanding differences in the SSR and isozyme profiles, the disclosure in the specification as well as the language of the claims is substantially similar to that of the instant application. Nevertheless, the examiner in Appeal No. 2005-0396 apparently found that appellant's specification provided an adequate written description of the claimed invention as no rejection of claims 6 and 11 was made under the written description provision of 35 U.S.C. § 112, first paragraph in Appeal No. 2005-0396. Accordingly, we find that the examiner has treated claims 6 and 11 in a manner that is inconsistent with the prosecution of similar claims in related application 10/077,589, which is the subject matter of Appeal No. 2005-0396.

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[17] The examiner has indicated that claim 5 is allowable. Answer, page 2.

For the foregoing reasons, we are not persuaded by the examiner's arguments.

Claims 27-30

According to the examiner (Answer, page 18), "[c]laims 27-30 are drawn towards I026458 plants further comprising a single locus conversion, or wherein the single locus was stably inserted into a corn genome by transformation." The examiner finds, however, that "the specification does not describe identified or isolated single loci for all corn plant traits." Id. More specifically, the examiner finds (id.), claims 27-30 "broadly encompass single loci that have not been discovered or isolated." To the extent that the examiner is asserting that appellant has not provided an enabling disclosure of single loci that have not been identified, we note that to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath.

Nevertheless, it may be that the examiner's concern (Answer, page 35), is that "single genes that alone govern 'yield enhancement' or 'enhanced yield stability' have not been discovered." In this regard, the examiner asserts (Answer, page 36), "the references cited in the specification do not describe isolated single genes or loci that confer yield enhancement or yield stability." Therefore, the examiner concludes (id.), "[a]ppellant cannot be in possession of plants further comprising single loci that have yet to be identified." The examiner, however, provides no evidence to support the assertion that a person

of ordinary skill in the art would not recognize that single loci for yield enhancement or yield stability are known in the art. In this regard, we note that appellant discloses (specification, page 31), "[m]any single locus traits have been identified ... examples of these traits include, but are not limited to, ... enhanced nutritional quality, industrial usage, yield stability, and yield enhancement." It appears that the examiner has overlooked appellant's assertion that single locus traits for yield stability and yield enhancement are well known in the art. To this end, we direct the examiner's attention to, for example, United States Patent No. 5,936,145 ('145)[18], issued August 10, 1999, which is prior to the filing date of the instant application. For clarity, we reproduce claims 8, 29 and 39 of the '145 patent below:

> 8. A corn plant having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.
>
> 29. The corn plant of claim 8, further comprising a single gene conversion.
>
> 39. The single gene conversion of the corn plant of claim 29, where the gene confers enhanced yield stability.

As we understand it, claim 39 of the '145 patent, is drawn to a corn plant which comprises a single gene conversion, wherein the gene confers enhanced yield stability. Thus, contrary to the examiner's assertion it appears, for example, that a single gene that confers enhanced yield stability was known in the art prior to the filing date of the instant application. We remind the examiner "a patent need

[18] We note that the assignee of the '145 patent is DeKalb Genetics Corporation. The assignee of the present application is Monsanto Company, the parent of wholly-owned subsidiary DeKalb Genetics Corporation.

not teach, and preferably omits, what is well known in the art." Hybritech Incorporated v. Monoclonal Antibodies, Inc. 802 F.2d 1367, 1385, 231 USPQ 81, 94 (Fed. Cir. 1986).

We remind the examiner that the inquiry into whether the description requirement is met must be determined on a case-by-case basis and is a question of fact. In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). A description as filed is presumed to be adequate; unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. See e.g., In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. Accordingly, it is the examiner who has the initial burden of establishing by a preponderance of evidence that a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. Wertheim, 541 F.2d at 263, 191 USPQ at 97. On this record, the examiner provides no evidence to support the assertion that single loci that govern, for example, yield enhancement or enhanced yield stability are not described.

For the foregoing reasons, we are not persuaded by the examiner's arguments.

Claim 31

Claim 31 is drawn to a method of producing an inbred corn plant derived from the corn variety I026458. The claimed method begins by crossing a plant of the corn variety I026458 with any other corn plant. The method requires that the

progeny corn plant be crossed either to itself, or with any other corn plant, and that the progeny of this cross be further crossed to itself, or with another corn plant, and so on throughout several generations. As we understand it, claim 31, in its simplest form, is directed to a method of using a plant of the corn variety I026458 to produce an inbred corn plant.

Nevertheless, the examiner finds (Answer, page 19), "[a] review of the claim indicates that hybrid progeny of corn plant I026458 are required to perform further crosses, and that progeny of subsequent generations can be further outcrossed with different corn plants." Therefore, the examiner concludes (id.), "[t]he hybrid progeny of corn plant I026458, and progeny plants of subsequent generations, are essential to operate the claimed method." As we understand the examiner's argument, not only does appellant have to provide a written description of the starting corn plant (I026458), but appellant also must look into the future to determine every other potential corn plant that someone may wish to cross with the I026458 corn variety, and provide written descriptive support for not only every other corn plant that could be crossed with I026458, but also the resulting progeny of each cross.

As set forth in Reiffin, the purpose of the written description requirement is to "ensure that the scope of the right to exclude, as set forth in the claims does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification." Here the method of producing an inbred corn plant requires a plant of the corn variety I026458 be used as the starting material. To that end, to satisfy the written description requirement, the inventor

"must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath. The examiner has indicated that a claim to a plant of the corn variety I026458 is allowable, see e.g., appellant's claim 5. Therefore, in our opinion, there can be no doubt that appellant was in possession of a plant of the corn variety I026458, in addition to a method of using that plant to cross with any other corn plant to produce an inbred corn plant as set forth in appellant's claim 31.

In our opinion, it matters not what the other corn plants are, or what the progeny of a cross between corn variety I026458 and some other corn plant represents. As the examiner explains (Answer, page 20), patentability of the method of claim 31 "does not lie in the method steps, which require the simple acts of crossing corn plants, allowing progeny seed to be produced, and growing progeny plants from the seed...." In our opinion, patentability of the method of claim 31 does not lie in the various other or second corn plants either. In our opinion, patentability of the method of claim 31 lies in the use of the corn variety I026458. Accordingly, for the foregoing reasons, it is our opinion that appellant has "convey[ed] with reasonable clarity to those skilled in the art that, as of the filing date sought, [they were] in possession of the invention," Vas-Cath (emphasis omitted).

Summary

For the foregoing reasons, we reverse the rejection of claims 6, 11, 24, 25 and 27-31 under the written description provision of 35 U.S.C. § 112, first paragraph.

Enablement:

Claims 27-30 stand rejected under the enablement provision of 35 U.S.C. § 112, first paragraph. The examiner finds (Answer, page 41), claims 27-30 "are broadly drawn towards inbred corn plant I026458 further defined as having a genome comprising any single locus conversion, encoding any trait; or wherein the single locus was stably inserted into a corn genome by transformation." The examiner presents several lines of argument under this heading. We take each in turn.

I. Retaining all the morphological and physiological traits of I026458:

According to the examiner (Answer, page 41, emphasis added), appellant's specification "does not teach any I026458 plants comprising a single locus conversion produced by backcrossing, wherein the resultant plant retains all of its morphological and physiological traits in addition to exhibiting the single trait conferred by the introduced single locus. With reference to Hunsperger, Kraft, and Eshed the examiner asserts (Answer, bridging sentence, pages 44-45), "[t]he rejection raises the issue of how linkage drag hampers the insertion of single genes alone into a plant by backcrossing, while recovering all of the original plant's genome."

We note, however, that claims 27-30 do not require that the single locus conversion plant retain all of the morphological and physiological traits of the parent plant in addition to exhibiting the single trait conferred by the introduction of the single loci. Nor do claims 27-30 require that the resultant plant retain all of the original plant's genome in addition to the single locus transferred into the inbred via the backcrossing technique. As appellant explains (specification, page 30, emphasis added),

> [t]he term single locus converted plant as used herein refers to those corn plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single locus transferred into the inbred via the backcrossing technique.

See also appellant's definition of single locus converted (conversion) plant at page 23 of the specification. We find nothing in the appellant's specification to indicate that the single locus converted plant retains all of the morphological and physiological traits, or all of the genome, of the parent plant in addition to the single locus transferred via the backcrossing technique. Accordingly, we disagree with the examiner's construction of claims 27-30 as "directed to exactly plant I026458 further comprising the single locus," which appears to disregard appellant's definition of a single locus converted plant. See Answer, page 46, emphasis added.

The examiner appears to appreciate (Answer, page 46) that appellant's specification provides an example of a converted plant. See e.g., specification, pages 35-36. However, for the foregoing reasons, we are not persuaded by the

examiner's assertion (Answer, page 46) that the specification provides "no indication that all of the morphological and physiological traits of [this converted] ... corn plant were recovered, and that only one single locus was transferred from the donor plant." To the contrary, the examiner provides no evidence that the converted plant exemplified in appellant's specification did not retain essentially all of the desired morphological and physiological characteristics of the inbred in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique.

Further, we recognize appellant's argument (Brief, page 29) that the examiner failed to establish a nexus between Hunsperger's discussion of petunias; Kraft's discussion of sugar beets; and Eshed's discussion of tomatoes, and the subject matter of the instant application - corn. Absent evidence to the contrary, we agree with appellant (id.), "[t]he [examiner's] indication[19] that the references concerning petunias, sugar beets and tomatoes apply to corn is made without any support." That the examiner has failed to identify (Answer, page 45) an example "in the prior art of plants in which linkage drag does not occur," does not mean that linkage drag is expected to occur in corn breeding, which according to appellant (Reply Brief, page 11) "is extremely advanced and well known in the art...." In this regard, we agree with appellant (Brief, page 30; Accord Reply Brief, page 11), the examiner has improperly placed the burden on appellant to demonstrate that the examiner's unsupported assertion is not true.

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[19] See Answer page 45, wherein the examiner asserts "[l]inkage drag appears to be a phenomenon that occurs in all plant types."

We remind the examiner, as set forth in <u>In re Wright</u>, 999 F.2d 1557, 1561-62, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993):

> When rejecting a claim under the enablement requirement of section 112, the PTO bears an initial burden of setting forth a reasonable explanation as to why it believes that the scope of protection provided by that claim is not adequately enabled by the description of the invention provided in the specification of the application; this includes, of course, providing sufficient reasons for doubting any assertions in the specification as to the scope of enablement.

II. <u>What plant is transformed in claim 28</u>?

We recognize the examiner's assertion (Answer, page 43) that while claim 28 requires that a single locus be stably inserted into a corn genome by transformation, the claim does not indicate whether (1) the I026458 plant was transformed with the single locus, or (2) some other corn plant was transformed with the single locus and then introduced into I026458 by crossing. However, as appellant points out (Brief, page 11), claim 28 "specifies that the single locus was stably inserted into a corn genome. Loci that are stably inserted into a corn genome are also stably inherited. Thus the single locus need not have been inserted into the genome of corn variety I026458." Accordingly, the I026458 plant may be transformed with the single locus, or another plant may be transformed with the single locus and then introduced into I026458 by crossing.

It may be that the examiner is concerned that by transforming a non-I026458 plant with a single locus and then introducing this locus into I026458 by crossing would result in a plant that does not retain <u>all</u> of the morphological and

physiological traits, or all of the genome, of the I026458 plant. For the foregoing reasons, however, this line of reasoning is not persuasive.

III. The single locus to be introduced:

The examiner finds (Answer, page 43), "the claims do not place any limit on the single locus to be introduced" into I026458 plants. The examiner recognizes, however, that "[t]he prior art shows that hundreds of nucleotide sequences encoding products that confer various types of plant traits have been isolated at the time the instant invention was filed." Id. In addition, the examiner recognizes (id.), "[o]ne skilled in the art can transform any of these isolated nucleotide sequences known in the prior art into a corn plant cell, and regenerate a transgenic plant from the transformed cell."

Nevertheless, the examiner finds (Answer, bridging sentence, pages 43-44), "[u]ndue experimentation would be required by one skilled in the art to isolate single loci that govern the traits encompassed by the claims." In this regard, the examiner asserts (Answer, page 45) that the claims broadly encompass corn plants comprising any type of single loci, including those that have not yet been identified or isolated. To the extent that the examiner is asserting that appellant has not provided an enabling disclosure of single loci that have not been identified, we note that enablement under 35 U.S.C. § 112, first paragraph is evaluated as of appellant's filing date. As set forth in Chiron Corp. v. Genentech Inc., 363 F.3d 1247, 1254, 70 USPQ2d 1321, 1325-26 (Fed. Cir. 2004), "a patent document cannot enable technology that arises after the date of application. The law does not expect an applicant to disclose knowledge

invented or developed after the filing date. Such disclosure would be impossible. See In re Hogan, 559 F.2d 595, 605-06 [194 USPQ 527] (CCPA 1977)."

The examiner's comment, however, may be directed to his assertion (Answer, page 43) that "isolated loci whose products confer yield enhancement or enhanced yield stability (recited in claim 30), are not known in the prior art." However, as discussed, supra, it appears that contrary to the examiner's assertion a single locus that confers the trait of, for example, yield enhancement was known in the art prior to the filing date of the instant invention. In addition, as discussed, supra, appellant's specification asserts that such traits were known in the art. See specification, page 31. Accordingly, as set forth in In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971), the burden is on

> the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure.

On this record, we find only the examiner's unsupported conclusions as to why the specification does not enable the claimed invention. We remind the examiner that nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples. Marzocchi, 439 F.2d at 223, 169 USPQ at 369. In the absence of an evidentiary basis to support the rejection, the examiner has not sustained his initial burden of establishing a prima facie case of non-enablement.

In this regard, we note that the burden of proof does not shift to appellant until the examiner first meets his burden. Marzocchi, 439 F.2d at 223-224, 169 USPQ at 369-370.

We also recognize the examiner's assertion (Answer, page 44) that claims 27-29 "encompass plants with single loci whose functions are unknown ... [or where] the effects of expression of the single locus on the traits expressed by I026458 are unknown." While this may be true, the examiner has not provided any evidence to suggest that it would require undue experimentation to obtain a single locus converted plant wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. See specification, page 23.

While it is not expressly stated in the text of the examiner's rejection, it may be that the examiner is concerned that the claims include inoperative embodiments. If so, the examiner is directed to Atlas Powder Co. v. E.I. DuPont De Nemours & Co., 750 F.2d 1569, 1576-77, 224 USPQ 409, 414 (Fed. Cir. 1984):

> Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. "It is not a function of the claims to specifically exclude ... possible inoperative substances...." In re Dinh-Nguyen, 492 F.2d 856, 859-59, 181 USPQ 46, 48 (CCPA 1974)(emphasis omitted). Accord, In re Geerdes, 491 F.2d 1260, 1265, 180 USPQ 789, 793 (CCPA 1974); In re Anderson, 471 F.2d 1237, 1242, 176 USPQ 331, 334-35 (CCPA 1971). Of course, if the number of inoperative combinations becomes significant, and in effect forces one of ordinary skill in the art to experiment unduly in order to practice the claimed invention, the claims might indeed be

invalid. *See* e.g., In re Cook, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971).

On this record, the examiner provides no evidence that the number of inoperative embodiments is so large that a person of ordinary skill in the art would have to experiment unduly to practice the claimed invention. To the contrary, the examiner recognizes (Answer, page 43) that "[t]he prior art shows that hundreds of nucleotide sequences encoding products that confer various types of plant traits have been isolated at the time the instant invention was filed"; and that "[o]ne skilled in the art can transform any of these isolated nucleotide sequences known in the prior art into a corn plant cell, and regenerate a transgenic plant from the transformed cell." Accordingly, we are not persuaded by the examiner's unsupported assertions.

For the foregoing reasons, we reverse the rejection of claims 27-30 under the enablement provision of 35 U.S.C. § 112, first paragraph.

SUMMARY

We reverse the rejection of claims 3, 6, 11, 14-20, and 27-30 under 35 U.S.C. § 112, second paragraph.

We reverse the rejection of claims 6, 11, 24, 25 and 27-31 under the written description provision of 35 U.S.C. § 112, first paragraph.

We reverse the rejection of claims 27-30 under the enablement provision of 35 U.S.C. § 112, first paragraph.

For the reasons set forth in n. 5 infra, we have not considered the merits of the rejection of claim 26 under the written description provision of 35 U.S.C. § 112, first paragraph.

REVERSED

Toni R. Scheiner
Administrative Patent Judge

Donald E. Adams
Administrative Patent Judge

Lora M. Green
Administrative Patent Judge

BOARD OF PATENT APPEALS AND INTERFERENCES

Robert E. Hanson
FULBRIGHT & JAWORSKI L.L.P.
A REGISTERED LIMITED LIABILITY PARTNERSHIP
600 CONGRESS AVENUE, SUITE 2400
AUSTIN, TX 78701

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*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-5,936,145	08-1999	Bradbury	
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
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	I	US-			
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	K	US-			
	L	US-			
	M	US-			

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NON-PATENT DOCUMENTS

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Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

US005936145A

United States Patent [19]

Bradbury

[11] **Patent Number: 5,936,145**

[45] **Date of Patent: Aug. 10, 1999**

[54] **INBRED CORN PLANT 87DIA4 AND SEEDS THEREOF**

[75] Inventor: Peter J. Bradbury, Madison, Wis.

[73] Assignee: DeKalb Genetics Corporation, DeKalb, Ill.

[21] Appl. No.: 09/017,996

[22] Filed: Feb. 3, 1998

Related U.S. Application Data

[60] Provisional application No. 60/037,305, Feb. 5, 1997.

[51] Int. Cl.^{6} A01H 5/00; A01H 4/00; A01H 1/00; C12N 5/04

[52] U.S. Cl. 800/320.1; 800/298; 800/275; 800/271; 800/301; 800/302; 800/303; 435/412; 435/424; 435/430; 435/430.1

[58] Field of Search 800/320.1, 298, 800/275, 271, 303, 274, 302; 435/172.3, 172.1, 412, 424, 430, 430.1

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Primary Examiner—Gary Benzion
Attorney, Agent, or Firm—Arnold, White & Durkee

[57] **ABSTRACT**

According to the invention, there is provided an inbred corn plant designated 87DIA4. This invention thus relates to the plants, seeds and tissue cultures of the inbred corn plant 87DIA4, and to methods for producing a corn plant produced by crossing the inbred plant 87DIA4 with itself or with another corn plant, such as another inbred. This invention further relates to corn seeds and plants produced by crossing the inbred plant 87DIA4 with another corn plant, such as another inbred, and to crosses with related species. This invention further relates to the inbred and hybrid genetic complements of the inbred corn plant 87DIA4, and also to the RFLP and genetic isozyme typing profiles of inbred corn plant 87DIA4.

39 Claims, No Drawings

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INBRED CORN PLANT 87DIA4 AND SEEDS THEREOF

The present application claims the priority of co-pending U.S. Provisional Patent Application Serial No. 60/037,305, filed Feb. 5, 1997, the entire disclosure of which is incorporated herein by reference without disclaimer.

BACKGROUND OF THE INVENTION

I. Technical Field of the Invention

The present invention relates to the field of corn breeding. In particular, the invention relates to the inbred corn seed and plant designated 87DIA4, and derivatives and tissue cultures of such inbred plant.

II. Description of the Background Art

The goal of field crop breeding is to combine various desirable traits in a single variety/hybrid. Such desirable traits include greater yield, better stalks, better roots, resistance to insecticides, herbicides, pests, and disease, tolerance to heat and drought, reduced time to crop maturity, better agronomic quality, and uniformity in germination times, stand establishment, growth rate, maturity, and fruit size.

Breeding techniques take advantage of a plant's method of pollination. There are two general methods of pollination: a plant self-pollinates if pollen from one flower is transferred to the same or another flower of the same plant. A plant cross-pollinates if pollen comes to it from a flower on a different plant.

Corn plants (*Zea mays L.*) can be bred by both self-pollination and cross-pollination. Both types of pollination involve the corn plant's flowers. Corn has separate male and female flowers on the same plant, located on the tassel and the ear, respectively. Natural pollination occurs in corn when wind blows pollen from the tassels to the silks that protrude from the tops of the ear shoot.

Plants that have been self-pollinated and selected for type over many generations become homozygous at almost all gene loci and produce a uniform population of true breeding progeny, a homozygous plant. A cross between two such homozygous plants produce a uniform population of hybrid plants that are heterozygous for many gene loci. Conversely, a cross of two plants each heterozygous at a number of gene loci produces a population of hybrid plants that differ genetically and are not uniform. The resulting non-uniformity makes performance unpredictable.

The development of uniform corn plant hybrids requires the development of homozygous inbred plants, the crossing of these inbred plants, and the evaluation of the crosses. Pedigree breeding and recurrent selection are examples of breeding methods used to develop inbred plants from breeding populations. Those breeding methods combine the genetic backgrounds from two or more inbred plants or various other broad-based sources into breeding pools from which new inbred plants are developed by selfing and selection of desired phenotypes. The new inbreds are crossed with other inbred plants and the hybrids from these crosses are evaluated to determine which of those have commercial potential.

The pedigree breeding method for single-gene traits involves crossing two genotypes. Each genotype can have one or more desirable characteristics lacking in the other; or, each genotype can complement the other. If the two original parental genotypes do not provide all of the desired characteristics, other genotypes can be included in the

breeding population. Superior plants that are the products of these crosses are selfed and selected in successive generations. Each succeeding generation becomes more homogeneous as a result of self-pollination and selection. Typically, this method of breeding involves five or more generations of selfing and selection: $S_1 \rightarrow S_2$; $S_2 \rightarrow S_3$; $S_3 \rightarrow S_4$; $S_4 \rightarrow S_5$, etc. After at least five generations, the inbred plant is considered genetically pure.

Backcrossing can also be used to improve an inbred plant. Backcrossing transfers a specific desirable trait from one inbred or other source to an inbred that lacks that trait. This can be accomplished for example by first crossing a superior inbred (A) (recurrent parent) to a donor inbred (non-recurrent parent), which carries the appropriate gene(s) for the trait in question. The progeny of this cross are then mated back to the superior recurrent parent (A) followed by selection in the resultant progeny for the desired trait to be transferred from the non-recurrent parent. After five or more backcross generations with selection for the desired trait, the progeny are heterozygous for loci controlling the characteristic being transferred, but are like the superior parent for most or almost all other genes. The last backcross generation would be selfed to give pure breeding progeny for the gene(s) being transferred.

A single cross hybrid corn variety is the cross of two inbred plants, each of which has a genotype which complements the genotype of the other. The hybrid progeny of the first generation is designated F_1. Preferred F_1 hybrids are more vigorous than their inbred parents. This hybrid vigor, or heterosis, is manifested in many polygenic traits, including markedly improved higher yields, better stalks, better roots, better uniformity and better insect and disease resistance. In the development of hybrids only the F_1 hybrid plants are sought. An F_1 single cross hybrid is produced when two inbred plants are crossed. A double cross hybrid is produced from four inbred plants crossed in pairs (A×B and C×D) and then the two F_1 hybrids are crossed again (A×B)×(C×D).

The development of a hybrid corn variety involves three steps: (1) the selection of plants from various germplasm pools; (2) the selfing of the selected plants for several generations to produce a series of inbred plants, which, although different from each other, each breed true and are highly uniform; and (3) crossing the selected inbred plants with unrelated inbred plants to produce the hybrid progeny (F_1). During the inbreeding process in corn, the vigor of the plants decreases. Vigor is restored when two unrelated inbred plants are crossed to produce the hybrid progeny (F_1). An important consequence of the homozygosity and homogeneity of the inbred plants is that the hybrid between any two inbreds is always the same. Once the inbreds that give a superior hybrid have been identified, hybrid seed can be reproduced indefinitely as long as the homogeneity of the inbred parents is maintained. Conversely, much of the hybrid vigor exhibited by F_1 hybrids is lost in the next generation (F_2). Consequently, seed from hybrid varieties is not used for planting stock. It is not generally beneficial for farmers to save seed of F_1 hybrids. Rather, farmers purchase F_1 hybrid seed for planting every year.

North American farmers plant over 70 million acres of corn at the present time and there are extensive national and international commercial corn breeding programs. A continuing goal of these corn breeding programs is to develop high-yielding corn hybrids that are based on stable inbred plants that maximize the amount of grain produced and minimize susceptibility to environmental stresses. To accomplish this goal, the corn breeder must select and develop superior inbred parental plants for producing hybrids.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a corn plant designated 87DIA4. Also provided are corn plants having all the physiological and morphological characteristics of corn plant 87DIA4.

The inbred corn plant of the invention may further comprise, or have, a cytoplasmic factor that is capable of conferring male sterility. Parts of the corn plant of the present invention are also provided, such as, e.g., pollen obtained from an inbred plant and an ovule of the inbred plant.

The invention also concerns seed of the corn plant 87DIA4, which has been deposited with the ATCC. The invention thus provides inbred corn seed designated 87DIA4, and having ATCC Accession No. 203192.

The inbred corn seed of the invention may be provided as an essentially homogeneous population of inbred corn seed designated 87DIA4.

Essentially homogeneous populations of inbred seed are those that consist essentially of the particular inbred seed, and are generally purified free from substantial numbers of other seed, so that the inbred seed forms between about 90% and about 100% of the total seed, and preferably, between about 95% and about 100% of the total seed. Most preferably, an essentially homogeneous population of inbred corn seed will contain between about 98.5%, 99%, 99.5% and about 100% of inbred seed, as measured by seed grow outs.

In any event, even if a population of inbred corn seed was found, for some reason, to contain about 50%, or even about 20% or 15% of inbred seed, this would still be distinguished from the small fraction of inbred seed that may be found within a population of hybrid seed, e.g., within a bag of hybrid seed. In such a bag of hybrid seed offered for sale, the Governmental regulations require that the hybrid seed be at least about 95% of the total seed. In the practice of the present invention, the hybrid seed generally forms at least about 97% of the total seed. In the most preferred practice of the invention, the female inbred seed that may be found within a bag of hybrid seed will be about 1% of the total seed, or less, and the male inbred seed that may be found within a bag of hybrid seed will be negligible, i.e., will be on the order of about a maximum of 1 per 100,000, and usually less than this value.

The population of inbred corn seed of the invention is further particularly defined as being essentially free from hybrid seed. The inbred seed population may be separately grown to provide an essentially homogeneous population of inbred corn plant designated 87DIA4.

In another aspect, the present invention provides for single gene converted plants of 87DIA4. The single transferred gene may preferably be a dominant or recessive allele. Preferably, the single transferred gene will confer such traits as male sterility, herbicide resistance, insect resistance, resistance for bacterial, fungal, or viral disease, male fertility, enhanced nutritional quality, and industrial usage. The single gene may be a naturally occurring maize gene or a transgene introduced through genetic engineering techniques.

In another aspect, the present invention provides a tissue culture of regenerable cells of inbred corn plant 87DIA4. The tissue culture will preferably be capable of regenerating plants having the physiological and morphological characteristics of the foregoing inbred corn plant, and of regenerating plants having substantially the same genotype as the foregoing inbred corn plant. Preferably, the regenerable cells in such tissue cultures will be embryos, protoplasts, meristematic cells, callus, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks or stalks. Still further, the present invention provides corn plants regenerated from the tissue cultures of the invention.

In yet another aspect, the present invention provides processes for preparing corn seed or plants, which processes generally comprise crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is the inbred corn plant designated 87DIA4. These processes may be further exemplified as processes for preparing hybrid corn seed or plants, wherein a first inbred corn plant is crossed with a second, distinct inbred corn plant to provide a hybrid that has, as one of its parents, the inbred corn plant 87DIA4.

In a preferred embodiment, crossing comprises planting, in pollinating proximity, seeds of the first and second parent corn plant, and preferably, seeds of a first inbred corn plant and a second, distinct inbred corn plant; cultivating or growing the seeds of said first and second parent corn plants into plants that bear flowers; emasculating the male flowers of the first or second parent corn plant, i.e., treating the flowers so as to prevent pollen production, in order to produce an emasculated parent corn plant; allowing natural cross-pollination to occur between the first and second parent corn plants; and harvesting the seeds from the emasculated parent corn plant. Where desired, the harvested seed is grown to produce a corn plant or hybrid corn plant.

The present invention also provides corn seed and plants produced by a process that comprises crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is the inbred corn plant designated 87DIA4. In one embodiment, corn plants produced by the process are first generation (F_1) hybrid corn plants produced by crossing an inbred in accordance with the invention with another, distinct inbred. The present invention further contemplates seed of an F_1 hybrid corn plant.

In certain exemplary embodiments, the invention provides an F_1 hybrid corn plant and seed thereof, which hybrid corn plant is designated 4033843, having 871DIA4 as one inbred parent.

In yet a further aspect, the invention provides an inbred genetic complement of the corn plant designated 87DIA4. The phrase "genetic complement" is used to refer to the aggregate of nucleotide sequences, the expression of which sequences defines the phenotype of, in the present case, a corn plant, or a cell or tissue of that plant. An inbred genetic complement thus represents the genetic make up of an inbred cell, tissue or plant, and a hybrid genetic complement represents the genetic make up of a hybrid cell, tissue or plant. The invention thus provides corn plant cells that have a genetic complement in accordance with the inbred corn plant cells disclosed herein, and plants, seeds and diploid plants containing such cells.

Plant genetic complements may be assessed by genetic marker profiles, and by the expression of phenotypic traits that are characteristic of the expression of the genetic complement, e.g., isozyme typing profiles. Thus, such corn plant cells may be defined as having an RFLP genetic marker profile in accordance with the profile shown in Table 8, or a genetic isozyme typing profile in accordance with the profile shown in Table 9, or having both an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 8 and 9.

In another aspect, the present invention provides hybrid genetic complements, as represented by corn plant cells, tissues, plants and seeds, formed by the combination of a haploid genetic complement of an inbred corn plant of the invention with a haploid genetic complement of a second corn plant, preferably, another, distinct inbred corn plant. In another aspect, the present invention provides a corn plant regenerated from a tissue culture that comprises a hybrid genetic complement of this invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. DEFINITIONS

Barren Plants: Plants that are barren, i.e., lack an ear with grain, or have an ear with only a few scattered kernels.

Cg: *Colletotrichum graminicola* rating. Rating times 10 is approximately equal to percent total plant infection.

CLN: Corn Lethal Necrosis (combination of Maize (Chlorotic Mottle Virus and Maize Dwarf Mosaic virus) rating: numerical ratings are based on a severity scale where 1=most resistant to 9=susceptible.

Cn: *Corynebacterium nebraskense* rating. Rating times 10 is approximately equal to percent total plant infection.

Cz: *Cercospora zeae-maydis* rating. Rating times 10 is approximately equal to percent total plant infection.

Dgg: *Diatraea grandiosella* girdling rating (values are percent plants girdled and stalk lodged).

Dropped Ears: Ears that have fallen from the plant to the ground.

Dsp: Diabrotica species root ratings (1=least affected to 9=severe pruning).

Ear-Attitude: The attitude or position of the ear at harvest scored as upright, horizontal, or pendant.

Ear-Cob Color: The color of the cob, scored as white, pink, red, or brown.

Ear-Cob Diameter: The average diameter of the cob measured at the midpoint.

Ear-Cob Strength: A measure of mechanical strength of the cobs to breakage, scored as strong or weak.

Ear-Diameter: The average diameter of the ear at its midpoint.

Ear-Dry Husk Color: The color of the husks at harvest scored as buff, red, or purple.

Ear-Fresh Husk The color of the husks 1 to 2 weeks after pollination scored as Color: green, red, or purple.

Ear-Husk Bract: The length of an average husk leaf scored as short, medium, or long.

Ear-Husk Cover: The average distance from the tip of the ear to the tip of the husks. Minimum value no less than zero.

Ear-Husk Opening: An evaluation of husk tightness at harvest scored as tight, intermediate, or open.

Ear-Length: The average length of the ear.

Ear-Number Per The average number of ears per plant. Stalk:

Ear-Shank The average number of internodes on the ear shank. Internodes:

Ear-Shank Length: The average length of the ear shank.

Ear-Shelling Percent: The average of the shelled grain weight divided by the sum of the shelled grain weight and cob weight for a single ear.

Ear-Silk Color: The color of the silk observed 2 to 3 days after silk emergence scored as green-yellow, yellow, pink, red, or purple.

Ear-Taper (Shape): The taper or shape of the ear scored as conical, semi-conical, or cylindrical.

Ear-Weight: The average weight of an ear.

Early Stand: The percent of plants that emerge from the ground as determined in the early spring.

ER: Ear rot rating (values approximate percent ear rotted).

Final Stand Count: The number of plants just prior to harvest.

GDUs to Shed: The number of growing degree units (GDUs) or heat units required for an inbred line or hybrid to have approximately 50 percent of the plants shedding pollen as measured from time of planting. Growing degree units are calculated by the Barger Method, where the heat units for a 24-hour period are calculated as GDUs=[Maximum daily temperature+Minimum daily temperature)/2]−50. The highest maximum daily temperature used is 86 degrees Fahrenheit and the lowest minimum temperature used is 50 degrees Fahrenheit. GDUs to shed is then determined by summing the individual daily values from planting date to the date of 50 percent pollen shed.

GDUs to Silk: The number of growing degree units for an inbred line or hybrid to have approximately 50 percent of the plants with silk emergence as measured from time of planting. Growing degree units are calculated by the same methodology as indicated in the GDUs to shed definition.

Hc2: *Helminthosporium carbonum* race 2 rating. Rating times 10 is approximately equal to percent total plant infection.

Hc3: *Helminthosporium carbonum* race 3 rating. Rating times 10 is approximately equal to percent total plant infection.

Hm: *Helminthosporium maydis* race 0 rating. Rating times 10 is approximately equal to percent total plant infection.

Ht1: *Helminthosporium turcicum* race 1 rating. Rating times 10 is approximately equal to percent total plant infection.

Ht2: *Helminthosporium turcicum* race 2 rating. Rating times 10 is approximately equal to percent total plant infection.

HtG: +=Presence of Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection. −=Absence of a Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection. +/−=Segregation of a Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection.

Kernel-Aleurone The color of the aleurone scored as white, pink, tan, brown, Color: bronze, red, purple, pale purple, colorless, or variegated.

Kernel-Cap Color: The color of the kernel cap observed at dry stage, scored as white, lemon-yellow, yellow or orange.

Kernel-Endosperm The color of the endosperm scored as white, pale yellow, or Color: yellow.

Kernel-Endosperm The type of endosperm scored as normal, waxy, or opaque. Type:

Kernel-Grade: The percent of kernels that are classified as rounds.

Kernel-Length: The average distance from the cap of the kernel to the pedicel.

Kernel-Number Per The average number of kernels in a single row. Row:

Kernel-Pericarp The color of the pericarp scored as colorless, red-white crown, tan, Color: bronze, brown, light red, cherry red, or variegated.

Kernel-Row The direction of the kernel rows on the ear scored as straight, Direction: slightly curved, spiral, or indistinct (scattered).

Kernel-Row Number: The average number of rows of kernels on a single ear.

Kernel-Side Color: The color of the kernel side observed at the dry stage, scored as white, pale yellow, yellow, orange, red, or brown.

Kernel-Thickness: The distance across the narrow side of the kernel.

Kernel-Type: The type of kernel scored as dent, flint, or intermediate.

Kernel-Weight: The average weight of a predetermined number of kernels.

Kernel-Width: The distance across the flat side of the kernel.

Kz: *Kabatiella zeae* rating. Rating times 10 is approximately equal to percent total plant infection.

Leaf-Angle: Angle of the upper leaves to the stalk scored as upright (0 to 30 degrees), intermediate (30 to 60 degrees), or lax (60 to 90 degrees).

Leaf-Color: The color of the leaves 1 to 2 weeks after pollination scored as light green, medium green, dark green, or very dark green.

Leaf-Length: The average length of the primary ear leaf.

Leaf-Longitudinal A rating of the number of longitudinal creases on the leaf surface 1 Creases: to 2 weeks after pollination. Creases are scored as absent, few, or many.

Leaf-Marginal A rating of the waviness of the leaf margin 1 to 2 weeks after Waves: pollination. Rated as none, few, or many.

Leaf-Number: The average number of leaves of a mature plant. Counting begins with the cotyledonary leaf and ends with the flag leaf.

Leaf-Sheath A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks Anthocyanin: after pollination, scored as absent, basal-weak, basal-strong, weak or strong.

Leaf-Sheath A rating of the pubescence of the leaf sheath. Ratings are taken 1 Pubescence: to 2 weeks after pollination and scored as light, medium, or heavy.

Leaf-Width: The average width of the primary ear leaf measured at its widest point.

LSS: Late season standability (values times 10 approximate percent plants lodged in disease evaluation plots).

Moisture: The moisture of the grain at harvest.

On1: *Ostrinia nubilalis* 1st brood rating (1=resistant to 9=susceptible).

On2: *Ostrinia nubilalis* 2nd brood rating (1=resistant to 9=susceptible).

Relative Maturity: A maturity rating based on regression analysis. The regression analysis is developed by utilizing check hybrids and their previously established day rating versus actual harvest moistures. Harvest moisture on the hybrid in question is determined and that moisture value is inserted into the regression equation to yield a relative maturity.

Root Lodging: Root lodging is the percentage of plants that root lodge. A plant is counted as root lodged if a portion of the plant leans from the vertical axis by approximately 30 degrees or more.

Seedling Color: Color of leaves at the 6 to 8 leaf stage.

Seedling Height: Plant height at the 6 to 8 leaf stage.

Seedling Vigor: A visual rating of the amount of vegetative growth on a 1 to 9 scale, where 1 equals best. The score is taken when the average entry in a trial is at the fifth leaf stage.

Selection Index: The selection index gives a single measure of hybrid's worth based on information from multiple traits. One of the traits that is almost always included is yield. Traits may be weighted according to the level of importance assigned to them.

Sr: *Sphacelotheca reiliana* rating is actual percent infection.

Stalk-Anthocyanin: A rating of the amount of anthocyanin pigmentation in the stalk. The stalk is rated 1 to 2 weeks after pollination as absent, basal-weak, basal-strong, weak, or strong.

Stalk-Brace Root The color of the brace roots observed 1 to 2 weeks after pollination Color: as green, red, or purple.

Stalk-Diameter: The average diameter of the lowest visible internode of the stalk.

Stalk-Ear Height: The average height of the ear measured from the ground to the point of attachment of the ear shank of the top developed ear to the stalk.

Stalk-Internode The direction of the stalk internode observed after pollination as Direction: straight or zigzag.

Stalk-Internode The average length of the internode above the primary ear. Length:

Stalk Lodging: The percentage of plants that did stalk lodge. Plants are counted as stalk lodged if the plant is broken over or off below the ear.

Stalk-Nodes With The average number of nodes having brace roots per plant. Brace Roots:

Stalk-Plant Height: The average height of the plant as measured from the soil to the tip of the tassel.

Stalk-Tillers: The percent of plants that have tillers. A tiller is defined as a secondary shoot that has developed as a tassel capable of shedding pollen.

Staygreen: Staygreen is a measure of general plant health near the time of black layer formation (physiological maturity). It is usually recorded at the time the ear husks of most entries within a trial have turned a mature color. Scoring is on a 1 to 9 basis where 1 equals best.

STR: Stalk rot rating (values represent severity rating of 1=25 percent of inoculated internode rotted to 9=entire stalk rotted and collapsed).

SVC: Southeastern Virus Complex combination of Maize Chlorotic Dwarf Virus and Maize Dwarf Mosaic Virus) rating; numerical ratings are based on a severity scale where 1=most resistant to 9=susceptible (1988 reactions are largely Maize Dwarf Mosaic Virus reactions).

Tassel-Anther Color: The color of the anthers at 50 percent pollen shed scored as green-yellow, yellow, pink, red, or purple.

Tassel-Attitude: The attitude of the tassel after pollination scored as open or compact.

Tassel-Branch Angle: The angle of an average tassel branch to the main stem of the tassel scored as upright (less than 30 degrees), intermediate (30 to 45 degrees), or lax (greater than 45 degrees).

Tassel-Branch The average number of primary tassel branches. Number:

Tassel-Glume Band: The closed anthocyanin band at the base of the glume scored as present or absent.

Tassel-Glume Color: The color of the glumes at 50 percent shed scored as green, red, or purple.

Tassel-Length: The length of the tassel measured from the base of the bottom tassel branch to the tassel tip.

Tassel-Peduncle The average length of the tassel peduncle, measured from the base Length: of the flag leaf to the base of the bottom tassel branch.

Tassel-Pollen Shed: A visual rating of pollen shed determined by tapping the tassel and observing the pollen flow of approximately five plants per entry. Rated on a 1 to 9 scale where 9=sterile, 1=most pollen.

Tassel-Spike Length: The length of the spike measured from the base of the top tassel branch to the tassel tip.

Test Weight: The measure of the weight of the grain in pounds for a given volume (bushel) adjusted to 15.5 percent moisture.

Yield: Yield of grain at harvest adjusted to 15.5 percents moisture.

II. OTHER DEFINITIONS

Allele is any of one or more alternative forms of a gene, all of which alleles relate to one trait or characteristic. In a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes.

Backcrossing is a process in which a breeder repeatedly crosses hybrid progeny back to one of the parents, for example, a first generation hybrid (F_1) with one of the parental genotypes of the F1 hybrid.

Chromatography is a technique wherein a mixture of dissolved substances are bound to a solid support followed by passing a column of fluid across the solid support and varying the composition of the fluid. The components of the mixture are separated by selective elution.

Crossing refers to the mating of two parent plants.

Cross-pollination refers to fertilization by the union of two gametes from different plants.

Diploid refers to a cell or organism having two sets of chromosomes.

Electrophoresis is a process by which particles suspended in a fluid are moved under the action of an electrical field, and thereby separated according to their charge and molecular weight. This method of separation is well known to those skilled in the art and is typically applied to separating various forms of enzymes and of DNA fragments produced by restriction endonucleases.

Emasculate refers to the removal of plant male sex organs.

Enzymes are organic catalysts that can exist in various forms called isozymes.

F_1 Hybrid refers to the first generation progeny of the cross of two plants.

Genetic Complement refers to an aggregate of nucleotide sequences, the expression of which sequences defines the phenotype in corn plants, or components of plants including cells or tissue.

Genotype refers to the genetic constitution of a cell or organism.

Haploid refers to a cell or organism having one set of the two sets of chromosomes in a diploid.

Isozymes are one of a number of enzymes which catalyze the same reaction(s) but differ from each other, e.g., in primary structure and/or electrophoretic mobility. The differences between isozymes are under single gene, codominant control. Consequently, electrophoretic separation to produce band patterns can be equated to different alleles at the DNA level. Structural differences that do not alter charge cannot be detected by this method.

Isozyme typing profile refers to a profile of band patterns of isozymes separated by electrophoresis that can be equated to different alleles at the DNA level.

Linkage refers to a phenomenon wherein alleles on the same chromosome tend to segregate together more often than expected by chance if their transmission was independent.

Marker is a readily detectable phenotype, preferably inherited in codominant fashion (both alleles at a locus in a diploid heterozygote are readily detectable), with no environmental variance component, i.e., heritability of 1.

87DIA4 refers to the corn plant from which seeds having ATCC Accession No. 203192 were obtained, as well as plants grown from those seeds.

Phenotype refers to the detectable characteristics of a cell or organism, which characteristics are the manifestation of gene expression.

Quantitative Trait Loci (QTL) refer to genetic loci that control to some degree numerically representable traits that are usually continuously distributed.

Regeneration refers to the development of a plant from tissue culture.

RFLP genetic marker profile refers to a profile of band patterns of DNA fragment lengths typically separated by agarose gel electrophoresis, after restriction endonuclease digestion of DNA.

Self-pollination refers to the transfer of pollen from the anther to the stigma of the same plant.

Single Gene Converted (Conversion) Plant refers to plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single gene transferred into the inbred via the backcrossing technique.

Tissue Culture refers to a composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

III. INBRED CORN PLANT 87DIA4

In accordance with one aspect of the present invention, there is provided a novel inbred corn plant, designated 87DIA4. Inbred corn plant 87DIA4 is a yellow, dent corn inbred that can be compared to inbred corn plants 2FACC, 3AZA1, and AQA3, all of which are proprietary inbreds of DEKALB Genetics Corporation. 87DIA4 differs significantly (at the 1%, 5%, or 10% level) from these inbred lines in several aspects (Table 1, Table 2, and Table 3).

TABLE 1

COMPARISON OF 87DIA4 WITH 2FACC

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
2FACC	0.4	0.6	29.5	62.0	23.9	67.1	1.3	1482.6	1481.5	5.8	77.4
DIFF	−0.1	−0.5	−5.2	0.4	−6.1	−10.1	−1.1	−119.5	−124.4	2.4	−12.3
# LOC	15	13	8	15	14	8	14	8	8	13	13
P VALUE	0.88	0.65	0.00**	0.84	0.00**	0.00**	0.40	0.00**	0.00**	0.36	0.01*

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

TABLE 2

COMPARISON OF 87DIA4 WITH 3AZA1

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
3AZA1	1.1	1.6	23.6	62.0	15.9	58.4	0.1	1322.6	1321.2	8.4	41.1
DIFF	−0.8	−1.5	0.7	0.4	1.9	−1.4	0.1	40.5	35.9	−0.2	24.0
# LOC	15	13	8	15	14	8	14	8	8	13	13
P VALUE	0.41	0.19	0.66	0.84	0.13	0.48	0.94	0.00**	0.03*	0.94	0.00**

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

TABLE 3

COMPARISON OF 87DIA4 WITH AQA3

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
AQA3	0.4	0.5	25.9	62.7	14.4	58.1	1.0	1356.2	1348.6	15.2	35.7
DIFF	−0.1	−0.4	−1.6	−0.3	3.3	−1.1	−0.8	6.9	8.5	−7.0	29.4

TABLE 3-continued

COMPARISON OF 87DIA4 WITH AQA3

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
# LOC	15	13	8	15	14	8	14	8	8	10	13
P VALUE	0.86	0.72	0.34	0.86	0.00**	0.58	0.56	0.64	0.61	0.01*	0.00**

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

A. ORIGIN AND BREEDING HISTORY

Inbred plant 87DIA4 was derived from the cross between a line derived from 2FACC and 3AZA1.

87DIA4 shows uniformity and stability within the limits of environmental influence for the traits described hereinafter in Table 4. 87DIA4 has been self-pollinated and ear-rowed a sufficient number of generations with careful attention paid to uniformity of plant type to ensure homozygosity and phenotypic stability. No variant traits have been observed or are expected in 87DIA4.

A deposit of 2500 seeds of plant designated 87DIA4 has been made with the American Type Culture Collection (ATCC), Rockville Pike, Bethesda, Md. on Sep. 11, 1998. Those deposited seeds have been assigned Accession No. 203192. The deposit was made in accordance with the terms and provisions of the Budapest Treaty relating to deposit of microorganisms and is made for a term of at least thirty (30) years and at least five (05) years after the most recent request for the furnishing of a sample of the deposit was received by the depository, or for the effective term of the patent, whichever is longer, and will be replaced if it becomes non-viable during that period.

Inbred corn plants can be reproduced by planting such inbred seeds, growing the resulting corn plants under self-pollinating or sib-pollinating conditions with adequate isolation using standard techniques well known to an artisan skilled in the agricultural arts. Seeds can be harvested from such a plant using standard, well known procedures.

The origin and breeding history of inbred plant 87DIA4 can be summarized as follows:

Summer 1988 The cross 2FACC and AQA3 was made. Both inbreds are proprietary to DEKALB Genetics Corporation.

Winter 1988 S0 seed was grown (nursery row 67-51).

Summer 1989 S1 seed was grown (nursery rows 4-25 to 4-38).

Winter 1989 S2 seed was grown ear-to-row (nursery row 649-62).

Summer 1990 S3 seed was grown ear-to-row (nursery row 130-15).

Winter 1990 S4 seed was grown ear-to-row (nursery row C23-23).

Summer 1991 S5 seed was grown ear-to-row (nursery row 222-67).

Summer 1992 S6 seed was grown ear-to-row (nursery row 418-56).

Summer 1993 S7 seed was grown ear-to-row (nursery rows 346-32 to 346-39). Seed from all rows was bulked to form 87DIA4.

B. PHENOTYPIC DESCRIPTION

In accordance with another aspect of the present invention, there is provided a corn plant having the physiological and morphological characteristics of corn plant 87DIA4. A description of the physiological and morphological characteristics of corn plant 87DIA114 is presented in Table 4.

TABLE 4

MORPHOLOGICAL TRAITS FOR THE 87DIA4 PHENOTYPE

CHARACTERISTIC	87DIA4	2FACC	3AZA1	AQA3
1. STALK				
Diameter (width) cm	1.9	2.2	2.0	2.2
Anthocyanin	Absent	Absent	Absent	Absent
Nodes with Brace Roots	1.4	1.9	2.0	1.5
Brace Root Color	Red	Purple	—	Green
Internode Direction	Straight	Straight	Straight	Straight

TABLE 4-continued

MORPHOLOGICAL TRAITS FOR THE 87DIA4 PHENOTYPE				
CHARACTERISTIC	87DIA4	2FACC	3AZA1	AQA3
Internode Length cm.	10.2	12.7	14.0	11.3
2. LEAF				
Color	Med Green	Med Green	Med Green	Med Green
Length cm.	68.0	71.3	66.2	67.9
Width cm.	10.0	8.7	7.9	8.3
Sheath Anthocyanin	Weak	Weak	Weak	Absent
Sheath Pubescence	Medium	Light	Medium	Medium
Marginal Waves	Few	Few	Few	Few
Longitudinal Creases	Absent	Absent	—	Few
3. TASSEL				
Attitude	Compact	Compact	—	Open
Length cm.	29.5	26.7	33.0	33.0
Spike Length cm.	19.5	19.3	24.4	23.1
Peduncle Length cm.	2.9	5.2	3.6	3.6
Branch Number	4.5	7.7	3.8	5.5
Anther Color	Red	Pink	Tan	Grn-Yellow
Glume Color	Green	Green	Green	Green
Glume Band	Absent	Absent	Absent	Absent
4. EAR				
Silk Color	Pink	Tan	Grn-Yellow	Grn-Yellow
Number Per Stalk	1.1	1.1	1.6	1.4
Position (attitude)	Upright	Upright	Pendant	Upright
Length cm.	15.6	13.9	16.4	16.3
Shape	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Diameter cm.	3.8	4.2	3.4	3.6
Weight gm.	99.1	116.3	89.8	93.1
Shank Length cm.	16.5	14.8	20.7	14.9
Husk Bract	Short	Short	Short	Short
Husk Cover cm.	3.4	6.6	1.9	3.2
Husk Opening	Tight	Intermediate	—	Intermediate
Husk Color Fresh	Green	Lt Green	Green	Lt Green
Husk Color Dry	Buff	Buff	Buff	Buff
Cob Diameter cm.	2.5	2.6	1.7	2.1
Cob Color	Red	Red	Red	Red
Shelling Percent	85.1	81.4	85.8	85.0
5. KERNEL				
Row Number	14.0	14.7	12.3	15.1
Number Per row	31.4	25.5	32.2	33.2
Row Direction	Curved	Curved	Curved	Curved
Type	Dent	Dent	Dent	Dent
Cap Color	Yellow	Yellow	Yellow	Lemon Yellow
Side Color	Yellow	Deep Yellow	Orange	Orange
Length (depth) mm.	10.2	10.7	9.2	9.6
Width mm.	8.1	8.1	7.3	7.1
Thickness	4.3	4.3	4.2	3.8
Weight of 1000K gm.	281.5	280.7	223.8	173.7
Endosperm Type	Normal	Normal	Normal	Normal
Endosperm Color	Yellow	Yellow	Yellow	Yellow

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

IV. ADDITIONAL INBRED CORN PLANTS

The inbred corn plant 171KI3 has been employed with the corn plant of the present invention in order to produce an exemplary hybrid. A description of the physiological and morphological characteristics of this corn plant is presented herein at Table 5. Additional information for this inbred corn plant is presented in co-pending U.S. patent application Ser. No. 08/795,403, filed Feb. 5, 1997, the disclosure of which application is specifically incorporated herein by reference.

TABLE 5

MORPHOLOGICAL TRAITS FOR THE 171KI3 PHENOTYPE				
CHARACTERISTIC	171KI3	01CSI2	01IBH2	3IIH6
1. STALK				
Diameter (width) cm.	2.2	2.4	2.1	2.3

TABLE 5-continued

MORPHOLOGICAL TRAITS FOR THE 171KI3 PHENOTYPE

CHARACTERISTIC	171KI3	01CSI2	01IBH2	3IIH6
Anthocyanin	Absent	Absent	Absent	Absent
Nodes With Brace Roots	0.9	1.8	1.3	0.7
Brace Root Color	Green	Green	Green	—
Internode Direction	Straight	Straight	Straight	Straight
Internode Length cm.	15.9	12.8	14.4	13.1
2. LEAF				
Color	Med Green	—	Med Green	Med Green
Width cm.	9.7	8.9	8.9	8.0
Marginal Waves	Few	Few	Few	Few
3. TASSEL				
Length cm.	42.6	31.2	33.6	35.3
Spike Length cm.	22.9	23.2	23.1	25.2
Peduncle Length cm.	9.6	3.9	8.2	7.6
Branch Number	9.1	7.4	7.8	12.9
Anther Color	Purple	Grn-Yellow	Grn-Yellow	—
Glume Color	Purple	Green	Green	—
Glume Band	Present	Absent	Absent	Absent
4. EAR	Pink	Pink	—	Red
Silk Color	1.0	1.0	1.0	1.4
Number Per Stalk	Upright	—	—	Upright
Position (attitude)	14.6	16.0	14.6	15.6
Length cm.	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Shape	4.0	3.8	4.0	3.9
Diameter cm.	104.9	100.6	103.2	107.6
Weight gm.	10.3	14.1	10.1	9.6
Shank Length cm.	Short	Short	Short	Short
Husk Bract	6.4	2.5	4.4	3.7
Husk Cover cm.	Green	Green	Green	Green
Husk Color Fresh	Buff	Buff	Buff	Buff
Husk Color Dry	2.3	2.4	2.3	2.1
Cob Diameter cm.	Red	—	Red	Red
Cob Color	87.7	80.6	89.0	83.3
Shelling Percent				
5. KERNEL	14.6	14.8	16.3	15.3
Row Number	32.1	27.1	29.3	29.7
Number Per Row	Curved	Curved	Curved	Curved
Row Direction	Dent	—	Dent	—
Type	Yellow	Yellow	Yellow	Yellow
Cap Color	Deep Yellow	—	Orange	
Side Color	11.1	9.4	10.9	10.3
Length (depth) mm.	7.8	8.0	7.4	7.8
Width mm.	3.9	5.2	4.4	4.2
Thickness	269.0	252.4	233.0	247.8
Weight of 1000K gm.	Normal	Normal	Normal	Normal
Endosperm Type	Yellow	Yellow	Yellow	Yellow
Endosperm Color				

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

V. SINGLE GENE CONVERSIONS

When the term inbred corn plant is used in the context of the present invention, this also includes any single gene conversions of that inbred. The term single gene converted plant as used herein refers to those corn plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single gene transferred into the inbred via the backcrossing technique. Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the inbred. The term backcrossing as used herein refers to the repeated crossing of a hybrid progeny back to one of the parental corn plants for that inbred. The parental corn plant which contributes the gene for the desired characteristic is termed the nonrecurrent or donor parent. This terminology refers to the fact that the nonrecurrent parent is used one time in the backcross protocol and therefore does not recur. The parental corn plant to which the gene or genes from the nonrecurrent parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol (Poehlman & Sleper, 1994; Fehr, 1987). In a typical backcross protocol, the original inbred of interest (recurrent parent) is crossed to a second inbred (nonrecurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a corn plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the

converted plant, in addition to the single transferred gene from the nonrecurrent parent.

The selection of a suitable recurrent parent is an important step for a successful backcrossing procedure. The goal of a backcross protocol is to alter or substitute a single trait or characteristic in the original inbred. To accomplish this, a single gene of the recurrent inbred is modified or substituted with the desired gene from the nonrecurrent parent, while retaining essentially all of the rest of the desired genetic, and therefore the desired physiological and morphological, constitution of the original inbred. The choice of the particular nonrecurrent parent will depend on the purpose of the backcross, one of the major purposes is to add some commercially desirable, agronomically important trait to the plant. The exact backcrossing protocol will depend on the characteristic or trait being altered to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele may also be transferred. In this instance it may be necessary to introduce a test of the progeny to determine if the desired characteristic has been successfully transferred.

Many single gene traits have been identified that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques. Single gene traits may or may not be transgenic, examples of these traits include but are not limited to, male sterility, waxy starch, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, male fertility, enhanced nutritional quality, industrial usage, yield stability and yield enhancement. These genes are generally inherited through the nucleus. Some known exceptions to this are the genes for male sterility, some of which are inherited cytoplasmically, but still act as single gene traits. Several of these single gene traits are described in U.S. Ser. No. 07/113,561, filed Aug. 25, 1993, the disclosure of which is specifically hereby incorporated by reference.

Direct selection may be applied where the single gene acts as a dominant trait. An example might be the herbicide resistance trait. For this selection process, the progeny of the initial cross are sprayed with the herbicide prior to the backcrossing. The spraying eliminates any plants which do not have the desired herbicide resistance characteristic, and only those plants which have the herbicide resistance gene are used in the subsequent backcross. This process is then repeated for all additional backcross generations.

The waxy characteristic is an example of a recessive trait. In this example, the progeny resulting from the first backcross generation (BC1) must be grown and selfed. A test is then run on the selfed seed from the BC1 plant to determine which BC1 plants carried the recessive gene for the waxy trait. In other recessive traits, additional progeny testing, for example growing additional generations such as the BC1S1 may be required to determine which plants carry the recessive gene.

VI. ORIGIN AND BREEDING HISTORY OF AN EXEMPLARY SINGLE GENE CONVERTED PLANT

85DGD1 MLms is a single gene conversion of 85DGD1 to cytoplasmic male sterility. 85DGD1 MLms was derived using backcross methods. 85DGD1 (a proprietary inbred of DEKALB Genetics Corporation) was used as the recurrent parent and MLms, a germplasm source carrying ML cytoplasmic sterility, was used as the nonrecurrent parent. The breeding history of the single gene converted inbred 85DGD1 MLms can be summarized as follows:

Hawaii Nurseries Planting Date Apr. 2, 1992 Made up S-O: Female row 585 male row 500

Hawaii Nurseries Planting Date Jul. 15, 1992 S-O was grown and plants were backcrossed times 85DGD1 (rows 444' 443)

Hawaii Nurseries Planting Date Bulked seed of the BC1 was grown and Nov. 18, 1992 backcrossed times 85DGD1 (rows V3-27' V3-26)

Hawaii Nurseries Planting Date Apr. 2, 1993 Bulked seed of the BC2 was gown and backcrossed times 85DGD1 (rows 37' 36)

Hawaii Nurseries Planting Date Jul. 14, 1993 Bulked seed of the BC3 was grown and backcrossed times 85DGD1 (rows 99' 98)

Hawaii Nurseries Planting Date Bulked seed of BC4 was grown and backcrossed Oct. 28, 1993 times 85DGD1 (rows KS-63' KS-62)

Summer 1994 A single ear of the BC5 was grown and backcrossed times 85DGD1 (MC94-822' MC94-822-7)

Winter 1994 Bulked seed of the BC6 was grown and backcrossed times 85DGD1 (3Q-1' 3Q-2)

Summer 1995 Seed of the BC7 was bulked and named 85DGD1 MLms.

VII. TISSUE CULTURE AND IN VITRO REGENERATION OF CORN PLANTS

A further aspect of the invention relates to tissue culture of corn plants designated 87DIA4. As used herein, the term "tissue culture" indicates a composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant. Exemplary types of tissue cultures are protoplasts, calli, plant clumps, and plant cells that are intact in plants or parts of plants, such as embryos, pollen, flowers, kernels, ears, cobs, leaves, husks, stalks, roots, root tips, anthers, silk and the like. In a preferred embodiment, tissue culture is embryos, protoplast, meristematic cells, pollen, leaves or anthers. Means for preparing and maintaining plant tissue culture are well known in the art. By way of example, a tissue culture comprising organs such as tassels or anthers, has been used to produce regenerated plants. (See, U.S. patent applications Ser. No. 07/992,637, filed Dec. 18, 1992 and 07/995,938, filed Dec. 21, 1992, now issued as U.S. Pat. No. 5,322,789, issued Jun. 21, 1994, the disclosures of which are incorporated herein by reference).

VIII. TASSEL/ANTHER CULTURE

Tassels contain anthers which in turn enclose microspores. Microspores develop into pollen. For anther/microspore culture, if tassels are the plant composition., they are preferably selected at a stage when the microspores are uninucleate, that is, include only one, rather than 2 or 3 nuclei. Methods to determine the correct stage are well known to those killed in the art and include mitramycin fluorescent staining (Pace et al., 1987), trypan blue (preferred) and acetocarmine squashing. The mid-uninucleate microspore stage has been found to be the developmental stage most responsive to the subsequent methods disclosed to ultimately produce plants.

Although microspore-containing plant organs such as tassels can generally be pretreated at any cold temperature below about 25° C., a range of 4 to 25° C. is preferred, and a range of 8 to 14° C. is particularly preferred. Although other temperatures yield embryoids and regenerated plants, cold temperatures produce optimum response rates compared to pretreatment at temperatures outside the preferred range. Response rate is measured as either the number of embryoids or the number of regenerated plants per number of microspores initiated in culture.

Although not required, when tassels are employed as the plant organ, it is generally preferred to sterilize their surface.

Following surface sterilization of the tassels, for example, with a solution of calcium hypochloride, the anthers are removed from about 70 to 150 spikelets (small portions of the tassels) and placed in a preculture or pretreatment medium. Larger or smaller amounts can be used depending on the number of anthers.

When one elects to employ tassels directly, tassels are preferably pretreated at a cold temperature for a predefined time, preferably at 10° C. for about 4 days. After pretreatment of a whole tassel at a cold temperature, dissected anthers are further pretreated in an environment that diverts microspores from their developmental pathway. The function of the preculture medium is to switch the developmental program from one of pollen development that of embryoid/callus development. An embodiment of such an environment in the form of a preculture medium includes a sugar alcohol, for example mannitol or sorbitol, inositol or the like. An exemplary synergistic combination is the use of mannitol at a temperature of about 10° C. for a period ranging from about 10 to 14 days. In a preferred embodiment, 3 ml of 0.3 M mannitol combined with 50 mg/l of ascorbic acid, silver nitrate and colchicine is used for incubation of anthers at 10° C. for between 10 and 14 days. Another embodiment is to substitute sorbitol for mannitol. The colchicine produces chromosome doubling at this early stage. The chromosome doubling agent is preferably only present at the preculture stage.

It is believed that the mannitol or other similar carbon structure or environmental stress induces starvation and functions to force microspores to focus their energies on entering developmental stages. The cells are unable to use, for example, mannitol as a carbon source at this stage. It is believed that these treatments confuse the cells causing them to develop as embryoids and plants from microspores. Dramatic increases in development from these haploid cells, as high as 25 embryoids in 10^4 microspores, have resulted from using these methods.

In embodiments where microspores are obtained from anthers, microspores can be released from the anthers into an isolation medium following the mannitol preculture step. One method of release is by disruption of the anthers, for example, by chopping the anthers into pieces with a sharp instrument, such as a razor blade, scalpel or Waring blender. The resulting mixture of released microspores, anther fragments and isolation medium are then passed through a filter to separate microspores from anther wall fragments. An embodiment of a filter is a mesh, more specifically, a nylon mesh of about 112 mm pore size. The filtrate which results from filtering the microspore-containing solution is preferably relatively free of anther fragments, cell walls and other debris.

In a preferred embodiment, isolation of microspores is accomplished at a temperature below about 25° C. and, preferably at a temperature of less than about 15° C. Preferably, the isolation media, dispersing tool (e.g., razor blade) funnels, centrifuge tubes and dispersing container (e.g., petri dish) are all maintained at the reduced temperature during isolation. The use of a precooled dispersing tool to isolate maize microspores has been reported (Gaillard et al., 1991).

Where appropriate and desired, the anther filtrate is then washed several times in isolation medium. The purpose of the washing and centrifugation is to eliminate any toxic compounds which are contained in the non-microspore part of the filtrate and are created by the chopping process. The centrifugation is usually done at decreasing spin speeds, for example, 1000, 750, and finally 500 rpms.

The result of the foregoing steps is the preparation of a relatively pure tissue culture suspension of microspores that are relatively free of debris and anther remnants.

To isolate microspores, an isolation media is preferred. An isolation media is used to separate microspores from the anther walls while maintaining their viability and embryogenic potential. An illustrative embodiment of an isolation media includes a 6 percent sucrose or maltose solution combined with an antioxidant such as 50 mg/l of ascorbic acid, 0.1 mg/l biotin and 400 mg/l of proline, combined with 10 mg/l of nicotinic acid and 0.5 mg/l $AgNO_3$. In another embodiment, the biotin and proline are omitted.

An isolation media preferably has a higher antioxidant level where used to isolate microspores from a donor plant (a plant from which a plant composition containing a microspore is obtained) that is field grown in contrast to greenhouse grown. A preferred level of ascorbic acid in an isolation medium is from about 50 mg/l to about 125 mg/l and, more preferably from about 50 mg/l to about 100 mg/l.

One can find particular benefit in employing a support for the microspores during culturing and subculturing. Any support that maintains the cells near the surface can be used. The microspore suspension is layered onto a support, for example by pipetting. There are several types of supports which are suitable and are within the scope of the invention. An illustrative embodiment of a solid support is a TRANSWELL® culture dish. Another embodiment of a solid support for development of the microspores is a bilayer plate wherein liquid media is on top of a solid base. Other embodiments include a mesh or a millipore filter. Preferably, a solid support is a nylon mesh in the shape of a raft. A raft is defined as an approximately circular support material which is capable of floating slightly above the bottom of a tissue culture vessel, for example, a petri dish, of about a 60 or 100 mm size, although any other laboratory tissue culture vessel will suffice. In an illustrative embodiment, a raft is about 55 mm in diameter.

Culturing isolated microspores on a solid support, for example, on a 10 mm pore nylon raft floating on 2.2 ml of medium in a 60 mm petri dish, prevents microspores from sinking into the liquid medium and thus avoiding low oxygen tension. These types of cell supports enable the serial transfer of the nylon raft with its associated microspore/embryoids ultimately to full strength medium containing activated charcoal and solidified with, for example, GELRITE™ (solidifying agent). The charcoal is believed to absorb toxic wastes and intermediaries. The solid medium allows embryoids to mature.

The liquid medium passes through the mesh while the microspores are retained and supported at the medium-air interface. The surface tension of the liquid medium in the petri dish causes the raft to float. The liquid is able to pass through the mesh: consequently, the microspores stay on top. The mesh remains on top of the total volume of liquid medium. An advantage of the raft is to permit diffusion of nutrients to the microspores. Use of a raft also permits transfer of the microspores from dish to dish during subsequent subculture with minimal loss, disruption or disturbance of the induced embryoids that are developing. The rafts represent an advantage over the multi-welled TRANSWELL® plates, which are commercially available from COSTAR, in that the commercial plates are expensive. Another disadvantage of these plates is that to achieve the serial transfer of microspores to subsequent media, the membrane support with cells must be peeled off the insert in the wells. This procedure does not produce as good a yield nor as efficient transfers, as when a mesh is used as a vehicle for cell transfer.

The culture vessels can be further defined as either (1) a bilayer 60 mm petri plate wherein the bottom 2 ml of medium are solidified with 0.7 percent agarose, overlaid with 1 mm of liquid containing the microspores; (2) a nylon mesh raft wherein a wafer of nylon is floated on 1.2 ml of medium and 1 ml of isolated microspores is pipetted on top; or (3) TRANSWELL® plates wherein isolated microspores are pipetted onto membrane inserts which support the microspores at the surface of 2 ml of medium.

After the microspores have been isolated, they are cultured in a low strength anther culture medium until about the 50 cell stage when they are subcultured onto an embryoid/callus maturation medium. Medium is defined at this stage as any combination of nutrients that permit the microspores to develop into embryoids or callus. Many examples of suitable embryoid/callus promoting media are well known to those skilled in the art. These media will typically comprise mineral salts, a carbon source, vitamins, growth regulations. A solidifying agent is optional. A preferred embodiment of such a media is referred to by the inventor as the "D medium" which typically includes 6N1 salts, $AgNO_3$ and sucrose or maltose.

In an illustrative embodiment, 1 ml of isolated microspores are pipetted onto a 10 mm nylon raft and the raft is floated on 1.2 ml of medium "D", containing sucrose or, preferably maltose. Both calli and embryoids can develop. Calli are undifferentiated aggregates of cells. Type I is a relatively compact, organized and slow growing callus. Type II is a soft, friable and fast-growing one. Embryoids are aggregates exhibiting some embryo-like structures. The embryoids are preferred for subsequent steps to regenerating plants. Culture medium "D" is an embodiment of medium that follows the isolation medium and replaces it. Medium "D" promotes growth to an embryoid/callus. This medium comprises 6N1 salts at ⅛ the strength of a basic stock solution, (major components) and minor components, plus 12 percent sucrose or, preferably 12 percent maltose, 0.1 mg/l B1, 0.5 mg/l nicotinic acid, 400 mg/l proline and 0.5 mg/l silver nitrate. Silver nitrate is believed to act as an inhibitor to the action of ethylene. Multi-cellular structures of approximately 50 cells each generally arise during a period of 12 days to 3 weeks. Serial transfer after a two week incubation period is preferred.

After the petri dish has been incubated for an appropriate period of time, preferably two weeks, in the dark at a predefined temperature, a raft bearing the dividing microspores is transferred serially to solid based media which promotes embryo maturation. In an illustrative embodiment, the incubation temperature is 30° C. and the mesh raft supporting the embryoids is transferred to a 100 mm petri dish containing the 6N1-TGR-4P medium, an "anther culture medium." This medium contains 6N1 salts, supplemented with 0.1 mg/l TIBA, 12 percent sugar (sucrose, maltose or a combination thereof), 0.5 percent activated charcoal, 400 mg/l proline, 0.5 mg/l B, 0.5 mg/l nicotinic acid, and 0.2 percent GELRITE™ (solidifying agent) and is capable of promoting the maturation of the embryoids. Higher quality embryoids, that is, embryoids which exhibit more organized development, such as better shoot meristem formation without precocious germination were typically obtained with the transfer to full strength medium compared to those resulting from continuous culture using only, for example, the isolated microspore culture (IMC) Medium "D." The maturation process permits the pollen embryoids to develop further in route toward the eventual regeneration of plants. Serial transfer occurs to full strength solidified 6N1 medium using either the nylon raft, the TRANSWELL® membrane or bilayer plates, each one requiring the movement of developing embryoids to permit further development into physiologically more mature structures.

In an especially preferred embodiment, microspores are isolated in an isolation media comprising about 6 percent maltose, cultured for about two weeks in an embryoid/calli induction medium comprising about 12 percent maltose and then transferred to a solid medium comprising about 12 percent sucrose.

At the point of transfer of the raft after about two weeks incubation, embryoids exist on a nylon support. The purpose of transferring the raft with the embryoids to a solidified medium after the incubation is to facilitate embryo maturation. Mature embryoids at this point are selected by visual inspection indicated by zygotic embryo-like dimensions and structures and are transferred to the shoot initiation medium. It is preferred that shoots develop before roots, or that shoots and roots develop concurrently. If roots develop before shoots, plant regeneration can be impaired. To produce solidified media, the bottom of a petri dish of approximately 100 mm is covered with about 30 ml of 0.2 percent GELRITE™ (solidifying agent) solidified medium. A sequence of regeneration media are used for whole plant formation from the embryoids.

During the regeneration process, individual embryoids are induced to form plantlets. The number of different media in the sequence can vary depending on the specific protocol used. Finally, a rooting medium is used as a prelude to transplanting to soil. When plantlets reach a height of about 5 cm, they are then transferred to pots for further growth into flowering plants in a greenhouse by methods well known to those skilled in the art.

Plants have been produced from isolated microspore cultures by methods disclosed herein, including self-pollinated plants. The rate of embryoid induction was much higher with the synergistic preculture treatment consisting of a combination of stress factors, including a carbon source which can be capable of inducing starvation, a cold temperature and colchicine, than has previously been reported. An illustrative embodiment of the synergistic combination of treatments leading to the dramatically improved response rate compared to prior methods, is a temperature of about 10° C., mannitol as a carbon source, and 0.05 percent colchicine.

The inclusion of ascorbic acid, an anti-oxidant, in the isolation medium is preferred for maintaining good microspore viability. However, there seems to be no advantage to including mineral salts in the isolation medium. The osmotic potential of the isolation medium was maintained optimally with about 6 percent sucrose, although a range of 2 percent to 12 percent is within the scope of this invention.

In an embodiment of the embryoid/callus organizing media, mineral salts concentration in IMC Culture Media "D" is (⅛x), the concentration which is used also in anther culture medium. The 6N1 salts major components have been modified to remove ammonium nitrogen. Osmotic potential in the culture medium is maintained with about 12 percent sucrose and about 400 mg/l proline. Silver nitrate (0.5 mg/l) was included in the medium to modify ethylene activity. The preculture media is further characterized by having a pH of about 5.7 to 6.0. Silver nitrate and vitamins do not appear to be crucial to this medium but do improve the efficiency of the response.

Whole anther cultures can also be used in the production of monocotyledonous plants from a plant culture system. There are some basic similarities of anther culture methods

and microspore culture methods with regard to the media used. A difference from isolated microspore cultures is that undisrupted anthers are cultured, so that a support, eg., a nylon mesh support, is not needed. The first step in developing the anther cultures is to incubate tassels at a cold temperature. A cold temperature is defined as less than about 25° C. More specifically, the incubation of the tassels is preferably performed at about 10° C. A range of 8 to 14° C. is also within the scope of the invention. The anthers are then dissected from the tassels, preferably after surface sterilization using forceps, and placed on solidified medium. An example of such a medium is designated by the inventors as 6N1-TGR-P4.

The anthers are then treated with environmental conditions that area combinations of stresses that are capable of diverting microspores from gametogenesis to embryogenesis. It is believed that the stress effect of sugar alcohols in the preculture medium, for example, mannitol, is produced by inducing starvation at the predefined temperature. In one embodiment, the incubation pretreatment is for about 14 days at 10° C. It was found that treating the anthers in addition with a carbon structure, an illustrative embodiment being a sugar alcohol, preferably, mannitol, produces dramatically higher anther culture response rates as measured by the number of eventually regenerated plants, than by treatment with either cold treatment or mannitol alone. These results are particularly surprising in light of teachings that cold is better than mannitol for these purposes, and that warmer temperatures interact with mannitol better.

To incubate the anthers, they are floated on a preculture medium which diverts the microspores from gametogenesis, preferably on a mannitol carbon structure,, more specifically, 0.3 M of mannitol plus 50 mg/l of ascorbic acid. 3 ml is about the total amount in a dish, for example, a tissue culture dish, more specifically, a 60 mm petri dish. Anthers are isolated from about 120 spikelets for one dish yields about 360 anthers.

Chromosome doubling agents can be used in the preculture media for anther cultures. Several techniques for doubling chromosome number (Jensen, 1974; Wan et al., 1989) have been described. Colchicine is one of the doubling agents. However, developmental abnormalities arising from in vitro cloning are further enhanced by colchicine treatments, and previous reports indicated that colchicine is toxic to microspores. The addition of colchicine in increasing concentrations during mannitol pretreatment prior to anther culture and microspore culture has achieved improved percentages.

An illustrative embodiment of the combination of a chromosome doubling agent and preculture medium is one which contains colchicine. In a specific embodiment, the colchicine level is preferably about 0.05 percent. The anthers remain in the mannitol preculture medium with the additives for about 10 days at 10° C. Anthers are then placed on maturation media, for example, that designated 6N1-TGR-P4, for 3 to 6 weeks to induce embryoids. If the plants are to be regenerated from the embryoids, shoot regeneration medium is employed, as in the isolated microspore procedure described in the previous sections. Other regeneration media can be used sequentially to complete regeneration of whole plants.

The anthers are then exposed to embryoid/callus promoting medium, for example, that designated 6N1-TGR-P4 to obtain callus or embryoids. The embryoids are recognized by identification visually of embryonic-like structures. At this stage, the embryoids are transferred serially to a series of regeneration media. In an illustrative embodiment, the shoot initiation medium comprises BAP (6-benzyl-aminopurine) and NAA (naphthalene acetic acid). Regeneration protocols for isolated microspore cultures and anther cultures are similar.

IX. OTHER CULTURES AND REGENERATION

The present invention contemplates a corn plant regenerated from a tissue culture of an inbred (e.g., 87DIA4) or hybrid plant (e.g., 4033843) of the present invention. As is well known in the art, tissue culture of corn can be used for the in vitro regeneration of a corn plant. By way of example, a process of tissue culturing and regeneration of corn is described in European Patent Application, publication 160, 390, the disclosure of which is incorporated by reference. Corn tissue culture procedures are also described in Green & Rhodes (1982) and Duncan et al., (1985). The study by Duncan et al. (1985) indicates that 97 percent of cultured plants produced calli capable of regenerating plants. Subsequent studies have shown that both inbreds and hybrids produced 91 percent regenerable calli that produced plants.

Other studies indicate that non-traditional tissues are capable of producing somatic embryogenesis and plant regeneration. See, e.g., Songstad et al. (1988); Rao et al. (1986); and Conger et al. (1987), the disclosures of which are incorporated herein by reference. Regenerable cultures may be initiated from immature embryos as described in PCT publication WO 95/06128, the disclosure of which is incorporated herein by reference.

Briefly, by way of example, to regenerate a plant of this invention, cells are selected following growth in culture. Where employed, cultured cells are preferably grown either on solid supports or in the form of liquid suspensions as set forth above. In either instance, nutrients are provided to the cells in the form of media, and environmental conditions are controlled. There are many types of tissue culture media comprising amino acids, salts, sugars, hormones and vitamins. Most of the media employed to regenerate inbred and hybrid plants have some similar components, the media differ in the composition and proportions of their ingredients depending on the particular application envisioned. For example, various cell types usually grow in more than one type of media, but exhibit different growth rates and different morphologies, depending on the growth media. In some media, cells survive but do not divide. Various types of media suitable for culture of plant cells have been previously described and discussed above.

An exemplary embodiment for culturing recipient corn cells in suspension cultures includes using embryogenic cells in Type II (Armstrong & Green, 1985; (Gordon-Kamm et al., 1990) callus, selecting for small (10 to 30 m) isodiametric, cytoplasmically dense cells, growing the cells in suspension cultures with hormone containing media, subculturing into a progression of media to facilitate development of shoots and roots, and finally, hardening the plant and readying it metabolically for growth in soil.

Meristematic cells (i.e., plant cells capable of continual cell division and characterized by an undifferentiated cytological appearance, normally found at growing points or tissues in plants such as root tips, stem apices, lateral buds, etc.) can be cultured.

Embryogenic calli are produced essentially as described in PCT Publication WO 95/06128. Specifically, inbred plants or plants from hybrids produced from crossing an inbred of the present invention with another inbred are grown to flowering in a greenhouse. Explants from at least one of the following F_1 tissues: the immature tassel tissue, intercalary meristems and leaf bases, apical meristems, immature ears and immature embryos are placed in an

initiation medium which contain MS salts, supplemented with thiamine, agar, and sucrose. Cultures are incubated in the dark at about 23° C. All culture manipulations and selections are performed with the aid of a dissecting microscope.

After about 5 to 7 days, cellular outgrowths are observed from the surface of the explants. After about 7 to 21 days, the outgrowths are subcultured by placing them into fresh medium of the same composition. Some of the intact immature embryo explants are placed on fresh medium. Several subcultures later (after about 2 to 3 months) enough material is present from explants for subdivision of these embryogenic calli into two or more pieces.

Callus pieces from different explants are not mixed. After further growth and subculture (about 6 months after embryogenic callus initiation), there are usually between 1 and 100 pieces derived ultimately from each selected explant. During this time of culture expansion, a characteristic embryogenic culture morphology develops as a result of careful selection at each subculture. Any organized structures resembling roots or root primordia are discarded. Material known from experience to lack the capacity for sustained growth is also discarded (translucent, watery, embryogenic structures). Structures with a firm consistency resembling at least in part the scutelum of the in vivo embryo are selected.

The callus is maintained on agar-solidified MS or N6-type media. A preferred hormone is 2,4-D. A second preferred hormone is dicamba. Visual selection of embryo-like structures is done to obtain subcultures. Transfer of material other than that displaying embryogenic morphology results in loss of the ability to recover whole plants from the callus.

Cell suspensions are prepared from the calli by selecting cell populations that appear homogeneous macroscopically. A portion of the friable, rapidly growing embryogenic calli is inoculated into MS or N6 Medium containing 2,4-D or dicamba. The calli in medium are incubated at about 27° C. on a gyrotary shaker in the dark or in the presence of low light. The resultant suspension culture is transferred about once every three to seven days, preferably every three to four days, by taking about 5 to 10 ml of the culture and introducing this inoculum into fresh medium of the composition listed above.

For regeneration, embryos which appear on the callus surface are selected and regenerated into whole plants by transferring the embryogenic structure, into a sequence of solidified media which include decreasing concentrations of 2,4-D or other auxins. Other hormones which can be used in culture media include dicamba, NAA, ABA, BAP, and 2-NCA. The reduction is relative to the concentration used in culture maintenance media. Plantlets are regenerated from these embryos by transfer to a hormone-free medium, subsequently transferred to soil, and grown to maturity.

Progeny are produced by taking pollen and selfing, backcrossing or sibling regenerated plants by methods well known to those skilled in the arts. Seeds are collected from the regenerated plants.

X. PROCESSES OF PREPARING CORN PLANTS AND THE CORN PLANTS PRODUCED BY SUCH CROSSES

The present invention also provides a process of preparing a novel corn plant and a corn plant produced by such a process. In accordance with such a process, a first parent corn plant is crossed with a second parent corn plant wherein at least one of the first and second corn plants is the inbred corn plant 87DIA4. In one embodiment, a corn plant prepared by such a process is a first generation F_1 hybrid corn plant prepared by a process wherein both the first and second parent corn plants are inbred corn plants.

Corn plants (*Zea mays L.*) can be crossed by either natural or mechanical techniques. Natural pollination occurs in corn when wind blows pollen from the tassels to the silks that protrude from the tops of the incipient ears. Mechanical pollination can be effected either by controlling the types of pollen that can blow onto the silks or by pollinating by hand.

In a preferred embodiment, crossing comprises the steps of:

(a) planting in pollinating proximity seeds of a first and a second parent corn plant, and preferably, seeds of a first inbred corn plant and a second, distinct inbred corn plant;

(b) cultivating or growing the seeds of the first and second parent corn plants into plants that bear flowers;

(c) emasculating flowers of either the first or second parent corn plant, i.e., treating the flowers so as to prevent pollen production, in order to produce an emasculated parent corn plant;

(d) allowing natural cross-pollination to occur between the first and second parent corn plant;

(e) harvesting seeds produced on the emasculated parent corn plant; and, where desired,

(f) growing the harvested seed into a corn plant, or preferably, a hybrid corn plant.

Parental plants are planted in pollinating proximity to each other by planting the parental plants in alternating rows, in blocks or in any other convenient planting pattern. Plants of both parental parents are cultivated and allowed to grow until the time of flowering. Advantageously, during this growth stage, plants are in general treated with fertilizer and, or other agricultural chemicals as considered appropriate by the grower.

At the time of flowering, in the event that plant 87DIA4, is employed as the male parent, the tassels of the other parental plant are removed from all plants employed as the female parental plant. The detasseling can be achieved manually but also can be done by machine, if desired.

The plants are then allowed to continue to grow and natural cross-pollination occurs as a result of the action of wind, which is normal in the pollination of grasses, including corn. As a result of the emasculation of the female parent plant, all the pollen from the male parent plant 87DIA4 is available for pollination because tassels, and thereby pollen bearing flowering parts, have been previously removed from all plants of the inbred plant being used as the female in the hybridization. Of course, during this hybridization procedure, the parental varieties are grown such that they are isolated from other corn fields to minimize or prevent my accidental contamination of pollen from foreign sources. These isolation techniques are well within the skill of those skilled in this art.

Both parental inbred plants of corn may be allowed to continue to grow until maturity or the male rows may be destroyed after flowering is complete. Only the ears from the female inbred parental plants are harvested to obtain seeds of a novel F_1 hybrid. The novel F_1 hybrid seed produced can then be planted in a subsequent growing season with the desirable characteristics in terms of F_1 hybrid corn plants providing improved grain yields and the other desirable characteristics disclosed herein, being achieved.

Alternatively, in another embodiment, both first and second parent corn plants can come from the same inbred corn plant, i.e., from the inbred designated 87DIA4. Thus, any corn plant produced using a process of the present invention and inbred corn plant 87DIA4, is contemplated by this invention. As used herein, crossing can mean selfing,

backcrossing, crossing to another or the same inbred, crossing to populations, and the like. All corn plants produced using the present inbred corn plant 87DIA4 as a parent are within the scope of this invention.

The utility of the inbred plant 87DIA4 also extends to crosses with other species. Commonly, suitable species will be of the family Graminaceae, and especially of the genera Zea, Tripsacum, Coix, Schlerachne, Polytoca, Chionachne, and Trilobachne, of the tribe Maydeae. Of these, Zea and Tripsacum, are most preferred. Potentially suitable for crosses with 87DIA4 can be the various varieties of grain sorghum, *Sorghum bicolor* (L.) Moench.

A. F_1 HYBRID CORN PLANT AND SEED PRODUCTION

Where the inbred corn plant 87DIA4 is crossed with another, different, corn inbred, a first generation (F_1) corn hybrid plant is produced. Both a F_1 hybrid corn plant and a seed of that F_1 hybrid corn plant are contemplated as aspects of the present invention.

Inbred 87DIA4 has been used to prepare an F_1 hybrid corn plant, designated 4033843.

The goal of a process of producing an F_1 hybrid is to manipulate the genetic complement of corn to generate new combinations of genes which interact to yield new, or improved traits (phenotypic characteristics). A process of producing an F_1 hybrid typically begins with the production of one or more inbred plants. Those plants are produced by repeated crossing of ancestrally related corn plants to try and concentrate certain genes within the inbred plants. The production of inbred 87DIA4 has been set forth hereinbefore.

Corn has a diploid phase which means two conditions of a gene (two alleles) occupy each locus (position on a chromosome). If the alleles are the same at a locus, there is said to be homozygosity. If they are different, there is said to be heterozygosity. In a completely inbred plant, all loci are homozygous. Because many loci when homozygous are deleterious to the plant, in particular leading to reduced vigor, less kernels, weak and/or poor growth, production of inbred plants is an unpredictable and arduous process. Under some conditions, heterozygous advantage at some loci effectively bars perpetuation of homozygosity.

Inbreeding requires coddling and sophisticated manipulation by human breeders. Even in the extremely unlikely event inbreeding rather than crossbreeding occurred in natural corn, achievement of complete inbreeding cannot be expected in nature due to well known deleterious effects of homozygosity and the large number of generations the plant would have to breed in isolation. The reason for the breeder to create inbred plants is to have a known reservoir of genes whose gametic transmission is at least somewhat predictable.

The development of inbred plants generally requires at least about 5 to 7 generations of selfing. Inbred plants are then cross-bred in an attempt to develop improved F_1 hybrids. Hybrids are then screened and evaluated in small scale field trials. Typically, about 10 to 15 phenotypic traits, selected for their potential commercial value, are measured. A selection index of the most commercially important traits is used to help evaluate hybrids. FACT, an acronym for Field Analysis Comparison Trial (strip trials), is an on-farm testing program employed by DEKALB Plant Genetics to perform the final evaluation of the commercial potential of a product.

During the next several years, a progressive elimination of hybrids occurs based on more detailed evaluation of their phenotype. Eventually, strip trials (FACT) are conducted to formally compare the experimental hybrids being developed with other hybrids, some of which were previously developed and generally are commercially successful. That is, comparisons of experimental hybrids are made to competitive hybrids to determine if there was any advantage to further commercial development of the experimental hybrids. Examples of such comparisons are presented in Section B, hereinbelow.

When the inbred parental plant 87DIA4 is crossed with another inbred plant to yield a hybrid (such as the hybrid 4033843), the original inbred can serve as either the maternal or paternal plant. For many crosses, the outcome is the same regardless of the assigned sex of the parental plants.

However, there is often one of the parental plants that is preferred as the maternal plant because of increased seed yield and production characteristics. Some plants produce tighter ear husks leading to more loss, for example due to rot. There can be delays in silk formation which deleteriously affect timing of the reproductive cycle for a pair of parental inbreds. Seed coat characteristics can be preferable in one plant. Pollen can be shed better by one plant. Other variables can also affect preferred sexual assignment of a particular cross.

B. F_1 HYBRID COMPARISONS

As mentioned in Section A, hybrids are progressively eliminated following detailed evaluations of their phenotype, including formal comparisons with other commercially successful hybrids. Strip trials are used to compare the phenotypes of hybrids grown in as many environments as possible. They are performed in many environments to assess overall performance of the new hybrids and to select optimum growing conditions. Because the corn is grown in close proximity, environmental factors that affect gene expression, such as moisture, temperature, sunlight and pests, are minimized. For a decision to be made that a hybrid is worth making commercially available, it is not necessary that the hybrid be better than all other hybrids. Rather, significant improvements must be shown in at least some, traits that would create improvements in some niches.

Examples of such comparative data are set forth hereinbelow in Table 6, which presents a comparison of performance data for the hybrid 4033843, a hybrid made with 87DIA4 as one parent, versus a selected hybrid of commercial value (DK442).

All the data in Table 6 represents results across years and locations for research and/or strip trials. The "NTEST" represents the number of paired observations in designated tests at locations around the United States.

TABLE 6

COMPARATIVE DATA FOR 4033843

HYBRID	NTEST	SI % C	YLD BU	MST PTS	STL %	RTL %	DRP %	FLSTD % M	SV RAT

TABLE 6-continued

COMPARATIVE DATA FOR 4033843										
4033843	R 93	110.3	156.3	19.5	5.2	1.4	0.1	101.0	4.1
DK442		99.0	147.7	19.8	7.1	5.2	0.1	100.9	4.1
DEV		11.3**	8.6**	0.1	-1.9**	-3.8**	0.0	0.2	-0.1

HYBRID	NTEST	ELSTD % M	PHT INCH	EHT INCH	BAR %	SG RAT	TST LBS	FGDU	ESTR DAYS
4033843	R 93	104.4	89.8	40.5		5.0	54.2	1214.0	94.0
DK442		102.9	90.2	43.9		3.1	53.4	1251.0	93.5
DEV		1.5+	-0.4	-3.0**		1.8**	0.8**	-36.9**	0.1

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
LEGEND ABBREVIATIONS:
HYBD = Hybrid
TEST = Research/FACT
SI % C = Selection Index (percent of check)
YLD BU = Yield (bushels/acre)
MST PTS = Moisture
STL % = Stalk Lodging (percent)
RTL % = Root Lodging (percent)
DRP % = Dropped Ears (percent)
FLSTD % M = Final Stand (percent of test mean)
SV RAT = Seedling Vigor Rating
ELSTD % M = Early Stand (percent of test mean)
PHT INCH = Plant Height (inches)
EHT INCH = Ear Height (inches)
BAR % = Barren Plants (percent)
SG RAT = Staygreen Rating
TST LBS = Test Weight (pounds)
FGDU = GDUs to Shed
ESTR DAYS = Estimated Relative Maturity (days)

As can be seen in Table 6, the hybrid 4033843 has significantly higher yield with comparable moisture content when compared to a successful commercial hybrid. Significant differences are also shown in Table 6 for many other traits.

C. PHYSICAL DESCRIPTION OF F_1 HYBRIDS

The present invention also provides F_1 hybrid corn plants derived from the corn plant 87DIA4. Physical characteristics of exemplary hybrids are set forth in Table 7, which concerns 4033843, which has 87DIA4 as one inbred parent. An explanation of terms used in Table 7 can be found in the Definitions, set forth herein above.

TABLE 7

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996

	CHARACTERISTIC	VALUE
1.	STALK	
	Diameter (width) cm	2.6
	Anthocyanin	Absent
	Nodes with Brace Roots	1.5
	Brace Root Color	Red
	Internode Direction	Straight
	Internode Length cm.	16.0
2.	LEAF	
	Color	Med Green
	Length cm.	79.9
	Width cm.	10.7
	Sheath Anthocyanin	Absent
	Sheath Pubescence	Medium
	Marginal Waves	Medium
	Longitudinal Creases	Few

TABLE 7-continued

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996

	CHARACTERISTIC	VALUE
3.	TASSEL	
	Attitude	Compact
	Length cm.	48.1
	Spike Length cm.	27.4
	Peduncle Length cm.	12.1
	Branch Number	7.5
	Anther Color	Red
	Glume Color	Purple
	Glume Band	Absent
4.	EAR	
	Silk Color	Tan
	Number Per Stalk	1.1
	Position (attitude)	Upright
	Length cm.	20.7
	Shape	Semi-conical
	Diameter cm.	4.7
	Weight gm.	222.8
	Shank Length cm.	18.7
	Husk Bract	Short
	Husk Opening	Open
	Husk Color Fresh	Green
	Husk Color Dry	Buff
	Cob Diameter cm.	2.4
	Cob Color	Red
	Shelling Percent	88.6
5.	KERNEL	
	Row Number	15.4
	Number Per row	42.6
	Row Direction	Straight
	Type	Dent

TABLE 7-continued

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996

CHARACTERISTIC	VALUE
Cap Color	Yellow
Side Color	Deep Yellow
Length (depth) mm.	12.0
Width mm.	7.9
Thickness	4.1
Weight of 1000K gm.	307.0
Endosperm Type	Normal
Endosperm Color	Yellow

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

XI. GENETIC COMPLEMENTS

In another aspect, the present invention provides a genetic complement of a plant of this invention. In one embodiment, therefore, the present invention contemplates an inbred genetic complement of the inbred corn plant designated 87DIA4. In another embodiment, the present invention contemplates a hybrid genetic complement formed by the combination of a haploid genetic complement from 87DIA4 and another haploid genetic complement. Means for determining a genetic complement are well-known in the art.

As used herein, the phrase "genetic complement" means an aggregate of nucleotide sequences, the expression of which sequences defines the phenotype of a corn plant or a cell or tissue of that plant. By way of example, a corn plant is genotyped to determine the array of the inherited markers it possesses. Markers are alleles at a single locus. They are preferably inherited in codominant fashion so that the presence of both alleles at a diploid locus is readily detectable, and they are free of environmental variation, i.e., their heritability is 1. This genotyping is preferably performed on at least one generation of the descendant plant for which the numerical value of the quantitative trait or traits of interest are also determined. The array of single locus genotypes is expressed as a profile of marker alleles, two at each locus. The marker allelic composition of each locus can be either homozygous or heterozygous. Homozygosity is a condition where both alleles at a locus are characterized by the same nucleotide sequence. Heterozygosity refers to different conditions of the gene at a locus. Markers that are used for purposes of this invention include restriction fragment length polymorphisms (RFLPs) and isozymes.

A plant genetic complement can be defined by genetic marker profiles that can be considered "fingerprints" of a genetic complement. For purposes of this invention, markers are preferably distributed evenly throughout the genome to increase the likelihood they will be near a quantitative trait loci (QTL) of interest (e.g., in tomatoes, Helentjaris et al., U.S. Pat. No. 5,385,835, Nienhuis et al., 1987). These profiles are partial projections of a sample of genes. One of the uses of markers in general is to exclude, or alternatively include, potential parents as contributing to offspring.

Phenotypic traits characteristic of the expression of a genetic complement of this invention are distinguishable by electrophoretic separation of DNA sequences cleaved by various restriction endonucleases. Those traits (genetic markers) are termed RFLPs (restriction fragment length polymorphisms).

Restriction fragment length polymorphisms (RFLPs) are genetic differences detectable by DNA fragment lengths, typically revealed by agarose gel electrophoresis, after restriction endonuclease digestion of DNA. There are large numbers of restriction endonucleases available, characterized by their nucleotide cleavage sites and their source, e.g., Eco RI. Variations in RFLPs result from nucleotide base pair differences which alter the cleavage sites of the restriction endonucleases, yielding different sized fragments.

Means for performing RFLP analyses are well known in the art. Restriction fragment length polymorphism analyses reported herein were conducted by Linkage Genetics. This service is available to the public on a contractual basis. Probes were prepared to the fragment sequences, these probes being complementary to the sequences thereby being capable of hybridizing to them under appropriate conditions well known to those ,,killed in the art. These probes were labeled with radioactive isotopes or fluorescent dyes for ease of detection. After the fragments were separated by size, they were identified by the probes. Hybridization with a unique cloned sequence permits the identification of a specific chromosomal region (locus). Because all alleles at a locus are detectable, RFLPs are codominant alleles, thereby satisfying a criteria for a genetic marker. They differ from some other types of markers, e.g., from isozymes, in that they reflect the primary DNA sequence, they are not products of transcription or translation. Furthermore, different RFLP genetic marker profiles result from different arrays of restriction endonucleases.

The RFLP genetic marker profile of each of the parental inbreds and exemplary resultant hybrids were determined. Because an inbred is essentially homozygous at all relevant loci, an inbred should, in almost all cases, have only one allele at each locus. In contrast, a diploid genetic marker profile of a hybrid should be the sum of those parents, e.g., if one inbred parent had the allele A at a particular locus, and the other inbred parent had B, the hybrid is AB by inference. Subsequent generations of progeny produced by selection and breeding are anticipated to be of genotype A, B, or AB for that locus position. When the F1 plant is used to produce an inbred, the locus should be either A or B for that position. Surprisingly, it has been observed that in certain instances, novel RFLP genotypes arise during the breeding process. For example, a genotype of C is observed at a particular locus position from the cross of parental inbreds with A and B at that locus. Such a novel RFLP genotype is observed for the 87DIA4, at least, for the RFLP markers M5213S and M8B2369S, as shown in Table 8. These novel RFLP markers further define the 87DIA4 inbred from the parental inbreds from which it was derived. An RFLP genetic marker profile of 87DIA4 is presented in Table 8.

TABLE 8

RFLP PROFILE OF 87DIA4

PROBE/ENZYME	87DIA4	2FACC	3A2A1	AQA3
M0264H	D	G	G	D
M0306H	A	A	—	A
M0445E	C	B	B	C
M1120S	F	—	D	F
M1234H	D	D	E	E
M1236H	A	A	—	A
M1238H	A	A	F	K
M1401E	C	C	C	A
M1406H	A	—	B	B
M1447H	B	B	E	B
M1B725E	B	B	C	C
M2239H	A	A	C	C
M2297H	A	A	E	A

TABLE 8-continued

RFLP PROFILE OF 87DIA4

PROBE/ENZYME	87DIA4	2FACC	3AZA1	AOA3
M2298E	C	B	C	C
M2402H	E	E	E	E
M3212S	A	A	B	A
M3247E	D	B	D	D
M3257S	B	B	B	B
M3296H	D	A	D	D
M3432H	A	I	A	A
M3446S	C	B	C	C
M3457E	E	E	E	E
M4386H	B	B	A	A
M4396H	H	H	F	F
M4444H	B	B	A	A
M4UMC19H	A	A	A	A
M4UMC31S	D	A	B	D
M5213S	B	A	B	A
M5295E	C	D	C	C
M5408H	A	A	A	A
M5579S	B	B	B	B
M5UMC95H	A	A	B	B
M6223E	C	C	C	C
M6252H	D	—	D	E
M6280H	E	E	A	A
M6373E	A	E	A	A
M7263E	A	C	A	A
M7391H	C	C	A	A
M7392S	C	C	B	C
M7455H	A	A	C	C
M8110S	C	C	C	C
M8114E	B	B	E	E
M8268H	B	B	B	B
M8585H	A	A	A	A
M8B2369S	B	D	B	D
M8UMC48E	C	C	C	C
M9209E	C	C	A	A
M9266S	A	A	C	C
M9B713S	A	A	B	B
M2UMC34H	D	D	D	—
M6UMC85H	A	A	A	—
M9UMC94H	E	E	B	—
M3UM121X	C	C	C	—
M0UMC130	C	H	—	—

*Probes used to detect RFLPs are from Linkage Genetics, 1515 West 2200 South, Suite C, Salt Lake City, Utah 84119.

Another aspect of this invention is a plant genetic complement characterized by a genetic isozyme typing profile. Isozymes are forms of proteins that are distinguishable, for example, on starch gel electrophoresis, usually by charge and/or molecular weight. The techniques and nomenclature for isozyme analysis are described in, Stuber et al. (1988), which is incorporated by reference.

A standard set of loci can be used as a reference set. Comparative analysis of these loci is used to compare the purity of hybrid seeds, to assess the increased variability in hybrids compared to inbreds, and to determine the identity of seeds, plants, and plant parts. In this respect, an isozyme reference set can be used to develop genotypic "fingerprints."

Table 9 lists the identifying numbers of the alleles at isozyme loci types, and represents the exemplary genetic isozyme typing profile for 87DIA4.

TABLE 9

ISOZYME PROFILE OF 87DIA4

	ISOZYME ALLELE			
LOCUS	87DIA4	2FACC	3AZA1	AOA3
Acph1	2	2	4	4
Adh1	4	4	4	4
Cat3	9	9	9	9
Got1	4	4	4	4
Got2	4	4	4	4
Got3	4	4	4	4
Idh1	4	4	4	4
Idh2	6	6	6	6
Mdh1	6	6	6*	6
Mdh2	3.5	3.5	6	3
Mdh3	16	16	16	16
Mdh4	12	12	12	12
Mdh5	12	12	12	12
Pgm1	9	9	9	9
Pgm2	4	4	4	4
6Pgd1	3.8	3.8	3.8	3.8
6Pgd2	5	5	5	5
Phi1	4	4	4	5

*Allele is probably a 6, but null cannot be ruled out.

The present invention also contemplates a hybrid genetic complement formed by the combination of a haploid genetic complement of the corn plant 87DIA4 with a haploid genetic complement of a second corn plant. Means for combining a haploid genetic complement from the foregoing inbred with another haploid genetic complement can be any method hereinbefore for producing a hybrid plant from 87DIA4. It is also contemplated that a hybrid genetic complement can be prepared using in vitro regeneration of a tissue culture of a hybrid plant of this invention.

A hybrid genetic complement contained in the seed of a hybrid derived from 87DIA4 is a further aspect of this invention. Exemplary hybrid genetic complements are the genetic complements of the hybrid 4033843.

Table 10 shows the identifying numbers of the alleles for the hybrid 4033843, which are exemplary RFLP genetic marker profiles for hybrids derived from the inbred of the present invention. Table 10 concerns 4033843, which has 87DIA4 as one inbred parent.

TABLE 10

RFLP PROFILE FOR 4033843

Probe/Enzyme Combination	Allelic Pair
M0264H	DH
M0306H	AA
M0445E	BC
M1120S	EF
M1234H	AD
M1238H	AE
M1401E	AC
M1406H	AB
M1447H	BB
M1B725E	BB
M2239H	AD
M2297H	AC
M2298E	CC
M2402H	EE
M3212S	AC
M3257S	AB
M3296H	CD
M3432H	AA
M3446S	CF
M3457E	EE
M4386H	BD

TABLE 10-continued

RFLP PROFILE FOR 4033843

Probe/Enzyme Combination	Allelic Pair
M4396E	HH
M4444H	AB
M4UMC19H	AA
M4UMC31S	AD
M5213S	AB
M5408H	AA
M6223E	BC
M6252H	AD
M6280H	EG
M6373E	AE
M7263E	AA
M7391H	AC
M7392S	AC
M7455H	AB
M8110S	AC
M8114E	BB
M8268H	BL
M8585H	AB
M8B2369S	BB
M8UMC48E	CC
M9209E	AC
M9266S	AA
M9B713S	AA
M2UMC34H	DF
M9UMC94H	EE
M3UM121X	CD
M0UMC130	CC

*Probes used to detect RFLPs are from Linkage Genetics, 1515 West 2200 South, Suite C, Salt Lake City, Utah 84119.

The exemplary hybrid genetic complements of hybrid 4033843 may also be assessed by genetic isozyme typing profiles using a standard set of loci as a reference, set, using, e.g., the same, or a different, set of loci to those described above. Table 11 lists the identifying numbers of the alleles at isozyme loci types and presents the exemplary genetic isozyme typing profile for the hybrid 4033843, which is an exemplary hybrid derived from the inbred of the present invention. Table 11 concerns 4033843, which has 87DIA4 as one inbred parent.

TABLE 11

ISOZYME GENOTYPE FOR HYBRID 4033843

LOCUS	ISOZYME ALLELES
Acph1	2
Adh1	4
Cat3	9
Got1	4
Got2	4
Got3	4
Idh1	4
Idh2	6
Mdh1	6
Mdh2	3.5
Mdh3	16
Mdh4	12
Mdh5	12
Pgm1	9
Pgm2	4
6-Pgd1	3.8
6-Pgd2	5
Phi1	4

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been

described in terms of the foregoing illustrative embodiments, it will be apparent to those of skill in the art that variations, changes, modifications and alterations may be applied to the composition, methods, and in the steps or in the sequence of steps of the methods described herein, without departing, from the true concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

Armstrong & Green, "Establishment and Maintenance of Friable Embryogenic Maize Callus and the Involvement of L-Proline," *Planta,* 164:207–214, 1985.

Conger et al., "Somatic Embryogenesis from Cultured Leaf Segments of *Zea Mays,*" *Plant Cell Reports,* 6:345–347, 1987.

Duncan et al., "The Production of Callus Capable of Plant Regeneration from Immature Embryos of Numerous *Zea Mays* Genotypes," *Planta,* 165:322–332, 1985.

Fehr (ed.), *Principles of Cultivar Development,* Vol. 1: Theory and Technique, pp. 360–376, 1987.

Gaillard et al., "Optimization of Maize Microspore Isolation and Culture Condition for Reliable Plant Regeneration," *Plant Cell Reports,* 10(2):55, 1991.

Gordon-Kamm et al., "Transformation of Maize Cells and Regeneration of Fertile Transgenic Plants," *The Plant Cell,* 6:603–618, 1990.

Green & Rhodes, "Plant Regeneration in Tissue Cultures of Maize," *Maize for Biological Research,* Plant Molecular Biology Association, pp. 367–372, 1992.

Jensen, "Chromosome Doubling Techniques in Haploids," Haploids and Higher Plants—*Advances and Potentials, Proceedings of the First International Symposium,* University of Guelph, Jun. 10–14, 1974.

Nienhuis et al., "Restriction Fragment Length Polymorphism Analysis of Loci Associated with Insect Resistance in Tomato," *Crop Science,* 27:797–803, 1987.

Pace et al., "Anther Culture of Maize and the Visualization of Embryogenic Microspores by Fluorescent Microscopy," *Theoretical and Applied Genetics,* 73:83 63–869, 1987.

Poehlman & Sleper (eds), *Breeding Field Crops,* 4th Ed., pp. 172–175, 1995.

Rao et al., "Somatic Embryogenesis in Glume Callus Cultures," *Maize Genetics Cooperation Newsletter,* Vol. 60, 1986.

Songstad et al. "Effect of 1-Aminocyclopropate-1-Carboxylic Acid, Silver Nitrate, and Norbornadiene on Plant Regeneration from Maize Callus Cultures," *Plant Cell Reports,* 7:262–265, 1988.

Stuber et al., "Techniques and scoring procedures for starch gel electrophoresis of enzymes of maize *C. Zea mays, L.,*" *Tech. Bull.,* N. Carolina Agric. Res. Serv., Vol. 286, 1988.

Wan et al., "Efficient Production of Doubled Haploid Plants Through Colchicine Treatment of Anther-

Derived Maize Callus," *Theoretical and Applied Genetics*, 77:889–892, 1989.

What is claimed is:

1. Inbred corn seed of the corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

2. The inbred corn seed of claim 1, further defined as an essentially homogeneous population of inbred corn seed designated 87DIA4.

3. The inbred corn seed of claim 1, further defined as essentially free from hybrid seed.

4. An inbred corn plant produced by growing the seed of an inbred corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

5. Pollen of the plant of claim 4.

6. An ovule of the plant of claim 4.

7. An essentially homogeneous population of corn plants produced by growing the seed of an inbred corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

8. A corn plant having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

9. The corn plant of claim 8, further comprising a cytoplasmic factor conferring male sterility.

10. A tissue culture of regenerable cells of inbred corn plaint 87DIA4, wherein the tissue regenerates plants having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

11. The tissue culture of claim 10, wherein the regenerable cells are embryos, meristematic cells, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks, stalks, or protoplasts or callus derived therefrom.

12. A corn plant regenerated from the tissue culture of claim 10, having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

13. An inbred corn plant cell of the corn plant of claim 4 having:

(a) an RFLP genetic marker profile in accordance with the profile shown in Table 8; or

(b) a genetic isozyme typing profile in accordance with the profile shown in Table 9.

14. The inbred corn plant cell of claim 13, having an RFLP genetic marker profile in accordance with the profile shown in Table 8.

15. The inbred corn plant cell of claim 13, having a genetic isozyme typing profile in accordance with the profile shown in Table 9.

16. The inbred corn plant cell of claim 13, having an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 8 and 9.

17. The inbred corn plant cell of claim 13, located within a corn plant or seed.

18. The inbred corn plant of claim 4 having:

(a) an RFLP genetic marker profile in accordance with the profile shown in Table 8; or

(b) a genetic isozyme typing profile in accordance with the profile shown in Table 9.

19. The inbred corn plant of claim 18, having an RFLP genetic marker profile in accordance with the profile shown in Table 8.

20. The inbred corn plant of claim 18, having a genetic isozyme typing profile in accordance with the profile shown in Table 9.

21. The inbred corn plant of claim 18, having an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 3 and 9.

22. A process of preparing corn seed, comprising crossing a first parent corn plant with a second parent corn plant, wherein said first or second corn plant is the inbred corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192, wherein seed is allowed to form.

23. The process of claim 22, further defined as a process of preparing hybrid corn seed, comprising crossing a first inbred corn plant with a second, distinct inbred corn plant, wherein said first or second corn plant is the inbred corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

24. The process of claim 23, wherein crossing comprises the steps of:

(a) planting in pollinating proximity seeds of said first and second inbred corn plants;

(b) cultivating the seeds of said first and second inbred corn plants into plants that bear flowers;

(c) emasculating the male flowers of said first or second inbred corn plant to produce an emasculated corn plant;

(d) allowing cross-pollination to occur between said first and second inbred corn plants; and

(e) harvesting seeds produced on said emasculated corn plant.

25. The process of claim 24, further comprising growing said harvested seed to produce a hybrid corn plant.

26. Hybrid corn seed produced by the process of claim 23.

27. A hybrid corn plant produced by the process of claim 25.

28. The hybrid corn plant of claim 27, wherein the plant is a first generation (F_1) hybrid corn plant.

29. The corn plant of claim 8, further comprising a single gene conversion.

30. The corn plant of claim 29, wherein the single gene was stably inserted into a corn genome by transformation.

31. The single gene conversion of the corn plant of claim 29, where the gene is a dominant allele.

32. The single gene conversion of the corn plant of claim 29, where the gene is a recessive allele.

33. The single gene conversion corn plant of claim 29, where the gene confers herbicide resistance.

34. The single gene conversion of the corn plant of claim 29, where the gene confers insect resistance.

35. The single gene conversion of the corn plant of claim 29, where the gene confers resistance to bacterial, fungal, or viral disease.

36. The single gene conversion of the corn plant of claim 29, wherein the gene confers male sterility.

37. The single gene conversion of the corn plant of claim 29, where the gene confers waxy starch.

38. The single gene conversion of the corn plant of claim 29, where the gene confers improved nutritional quality.

39. The single gene conversion of the corn plant of claim 29, where the gene confers enhanced yield stability.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145
DATED : August 10, 1999
INVENTOR(S) : Peter J. Bradbury

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 5, at lines 46-47, please delete "Ear-Fresh Husk The color of the husks 1 to 2 weeks after pollination scored as Color: green, red, or purple" and substitute therefor --Ear-Fresh Husk Color: The color of the husks 1 to 2 weeks after pollination scored as green, red, or purple--.

In col. 5, at lines 56-57, please delete "Ear-Number Per The average number of ears per plant Stalk:" and substitute therefor --Ear-Number Per Stalk: The average number of ears per plant--.

In col. 5, at lines 58-59, please delete "Ear-Shank The average number of internodes on the ear shank. Internodes:" and substitute therefor --Ear-Shank Internodes: The average number of internodes on the ear shank--.

In col. 6, at lines 51-53, please delete "Kernel-Aleurone The color of the aleurone scored as white, pink, tan, brown, Color: bronze, red, purple, pale purple, colorless, or variegated" and substitute therefor --Kernel-Aleurone Color: The color of the aleurone scored as white, pink, tan, brown, bronze, red, purple, pale purple, colorless, or variegated--.

In col. 6, at lines 57-58, please delete "Kernel-Endosperm The color of the endosperm scored as white, pale yellow, or Color: yellow" and substitute therefor --Kernel-Endosperm Color: The color of the endosperm scored as white, pale yellow, or yellow--.

In col. 6, at lines 59-60, please delete "Kernel-Endosperm The type of endosperm scored as normal, waxy, or opaque. Type:" and substitute therefor --Kernel-Endosperm Type: The type of endosperm scored as normal, waxy, or opaque--.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 6, at lines 65-66, please delete "Kernel-Number Per The average number of kernels in a single row. Row:" and substitute therefor --Kernel-Number Per Row: The average number of kernels in a single row--.

In col. 7, at lines 1-3, please delete "Kernel-Pericarp The color of the pericarp scored as colorless, red-white crown, tan, Color: bronze, brown, light red, cherry red, or variegated" and substitute therefor -- Kernel-Pericarp Color: The color of the pericarp scored as colorless, red-white crown, tan, bronze, brown, light red, cherry red, or variegated--.

In col. 7, at lines 4-6, please delete "Kernel-Row The direction of the kernel rows on the ear scored as straight, Direction: slightly curved, spiral, or indistinct (scattered)" and substitute therefor --Kernel-Row Direction: The direction of the kernel rows on the ear scored as straight, slightly curved, spiral, or indistinct (scattered)--.

In col. 7, at lines 30-33, please delete "Leaf-Longitudinal A rating of the number of longitudinal creases on the leaf surface 1 Creases: to 2 weeks after pollination. Creases are scored as absent, few, or many" and substitute therefor --Leaf-Longitudinal Creases: A rating of the number of longitudinal creases on the leaf surface 1 to 2 weeks after pollination. Creases are scored as absent, few, or many--.

In col. 7, at lines 34-36, please delete "Leaf-Marginal A rating of the waviness of the leaf margin 1 to 2 weeks after Waves: pollination. Rated as none, few, or many" and substitute therefor --Leaf-Marginal Waves: A rating of the waviness of the leaf margin 1 to 2 weeks after pollination. Rated as none, few, or many--.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 7, at lines 40-43, please delete "Leaf-Sheath A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks Anthocyanin: after pollination, scored as absent, basal-weak, basal-strong, weak or strong" and substitute therefor --Leaf-Sheath Anthocyanin: A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks after pollination, scored as absent, basal-weak, basal-strong, weak or strong--.

In col. 7, at lines 44-46, please delete "Leaf-Sheath A rating of the pubescence of the leaf sheath. Ratings are taken 1 Pubescence: to 2 weeks after pollination and scored as light, medium, or heavy" and substitute therefor --Leaf-Sheath Pubescence: A rating of the pubescence of the leaf sheath. Ratings are taken 1 to 2 weeks after pollination and scored as light, medium, or heavy--.

In col. 8, at lines 19-21, please delete "Stalk-Brace Root The color of the brace roots observed 1 to 2 weeks after pollination Color: as green, red, or purple" and substitute therefor --Stalk-Brace Root Color: The color of the brace roots observed 1 to 2 weeks after pollination as green, red, or purple--.

In col. 8, at lines 27-29, please delete "Stalk-Internode The direction of the stalk internode observed after pollination as Direction: straight or zigzag" and substitute therefor --Stalk-Internode Direction: The direction of the stalk internode observed after pollination as straight or zigzag--.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 8, at lines 30-31, please delete "Stalk-Internode The average length of the internode above the primary ear. Length:" and substitute therefor --Stalk Internode Length: The average length of the internode above the primary ear--.

In col. 8, at lines 35-36, please delete "Stalk-Internode With The average number of nodes having brace roots per plant. Brace Roots:" and substitute therefor --Stalk-Internode With Brace Roots: The average number of nodes having brace roots per plant--.

In col. 8, at lines 65-66, please delete "Tassel-Branch The average number of primary tassel branches. Number:" and substitute therefor --Tassel-Branch Number: The average number of primary tassel branches--.

In col. 9, at lines 7-9, please delete "Tassel-Peduncle The average length of the tassel peduncle, measured from the base of the flag leaf to the base Length: of the bottom tassel branch" and substitute therefor --Tassel-Peduncle Length: The average length of the tassel peduncle, measured from the base of the flag leaf to the base of the bottom tassel branch--.

In col. 13, at line 26, delete "3AZA1" and substitute therefor --AQA3--.

In col. 14, at line 52, delete "87DIA114" and substitute therefor --87DIA4--.

In col. 30, at line 4, , delete "DEKALB Plant Genetics" and substitute therefor --DEKALB Genetics Corporation--.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At col. 17, in Table 5 delete all rows under "4. EAR" and ending with "Endosperm Color" and subsitute therefor the following rows --

4. EAR				
Silk Color	Pink	Pink	—	Red
Number Per Stalk	1.0	1.0	1.0	1.4
Position (attitude)	Upright	—	—	Upright
Length cm.	14.6	16.0	14.6	15.6
Shape	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Diameter cm.	4.0	3.8	4.0	3.9
Weight gm.	104.9	100.6	103.2	107.6
Shank Length cm.	10.3	14.1	10.1	9.6
Husk Bract	Short	Short	Short	Short
Husk Cover cm.	6.4	2.5	4.4	3.7
Husk Color Fresh	Green	Green	Green	Green
Husk Color Dry	Buff	Buff	Buff	Buff
Cob Diameter cm.	2.3	2.4	2.3	2.1
Cob Color	Red	—	Red	Red
Shelling Percent	87.7	80.6	89.0	83.3

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145
DATED : August 10, 1999
INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

5. KERNEL

Row Number	14.6	14.8	16.3	15.3
Number Per Row	32.1	27.1	29.3	29.7
Row Direction	Curved	Curved	Curved	Curved
Type	Dent	—	Dent	—
Cap Color	Yellow	Yellow	Yellow	Yellow
Side Color	Deep Yellow	—	Orange	
Length (depth) mm.	11.1	9.4	10.9	10.3
Width mm.	7.8	8.0	7.4	7.8
Thickness	3.9	5.2	4.4	4.2
Weight of 1000K gm.	269.0	252.4	233.0	247.8
Endosperm Type	Normal	Normal	Normal	Normal
Endosperm Color	Yellow	Yellow	Yellow	Yellow--.

Signed and Sealed this

Twenty-seventh Day of February, 2001

Attest:

Nicholas P. Godici

NICHOLAS P. GODICI

Attesting Officer

Acting Director of the United States Patent and Trademark Office

EXHIBIT F

The opinion in support of the decision being entered today was not written for publication and is not binding precedent of the Board.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte Marvin L. Boerboom

Appeal No. 2005-0396[1]
Application No. 10/077,589

ON BRIEF[2]

Before SCHEINER, ADAMS and GREEN, Administrative Patent Judges.

ADAMS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on the appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 3, 6, 11, 15-20 and 24-31. The examiner has indicated that claims 1, 2, 5, 7-10, 12-14 and 21-23 are allowable. Answer, page 2. Claim 4 has been cancelled. Id.

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[1] This appeal is substantially similar to Appeal No. 2004-1503, Application No. 09/606,808; Appeal No. 2004-1506, Application No. 09/788,334; Appeal No. 2004-1968, Application No. 10/000,311; Appeal No. 2004-2317, Application No. 09/771,938; and Appeal No. 2004-2343, Application No. 09/772,520, which all share the same assignee, Monsanto Company, the parent of wholly-owned subsidiary DeKalb Genetics Corporation. Accordingly we have considered these appeals together.

[2] We note that appellant waived his request for Oral Hearing. See Paper received January 5, 2005.

Claims 3, 6, 15, 16, 17, 27, 28 and 30 are illustrative of the subject matter on appeal and are reproduced below. In addition, for convenience, we have reproduced allowable claims 2 and 5 below:

2. A population of seed of the corn variety I180580, wherein a sample of the seed of the corn variety I180580 was deposited under ATCC Accession No. PTA-4490.[3]

3. The population of seed of claim 2, further defined as an essentially homogeneous population of seed.

5. A corn plant produced by growing a seed of the corn vadety I180580, wherein a sample of the seed of the corn variety I180580 was deposited under ATCC Accession No. PTA-4490.

6. The corn plant of claim 5, having:
   (a) an SSR profile in accordance with the profile shown in Table 6; or
   (b) an isozyme typing profile in accordance with the profile shown in Table 7.[4]

15. A corn plant capable of expressing all the physiological and morphological characteristics of the corn variety I180580, wherein a sample of the seed of the corn variety I180580 was deposited under ATCC Accession No. PTA-4490.

16. The corn plant of claim 15, further comprising a nuclear or cytoplasmic gene conferring male sterility.

17. A tissue culture of regenerable cells of a plant of corn variety I180580, wherein the tissue is capable of regenerating plants capable of expressing all the physiological and morphological characteristics of the corn variety I180580, wherein a sample of the seed of the corn variety I180580 was deposited under ATCC Accession No. PTA-4490.

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[3] The appendix of claims in appellant's Brief incorrectly identifies the ATCC Accession No. as PTA-3224. However, according to appellant's Declaration of Biological Deposit, received April 21, 2003, the ATCC Accession No. for seeds of corn inbred I180580 is PTA-4490. Accordingly, all references, herein, to the deposited seeds will refer to the correct ATCC Accession No. PTA-4490.

[4] The appendix of claims in appellant's Brief incorrectly refers to the SSR profile of "Table 5," and the isozyme profile of "Table 6". As we understand appellant's specification (pages 59-62), the SSR profile is shown in Table 6, and the isozyme profile is shown in Table 7. Accordingly, all references, herein, to the SSR and isozyme profiles refer Tables 6 and 7 respectively.

27. The corn plant of claim 5, further defined as having a genome comprising a single locus conversion.

28. The corn plant of claim 27, wherein the single locus was stably inserted into a corn genome by transformation.

30. The corn plant of claim 27, wherein the locus confers a trait selected from the group consisting of herbicide tolerance; insect resistance; resistance to bacterial, fungal, nematode or viral disease; yield enhancement; waxy starch; improved nutritional quality; enhanced yield stability; male sterility and restoration of male fertility.

The references relied upon by the examiner are:

Hunsperger et al. (Hunsperger)	5,523,520	Jun. 4, 1996

Eshed et al. (Eshed), "Less-Than-Additive Epistatic Interactions of Quantitative Trait Loci in Tomato," Genetics, Vol. 143, pp. 1807-17 (1996)

Kraft et al. (Kraft), "Linkage Disequilibrium and Fingerprinting in Sugar Beet," Theoretical and Applied Genetics, Vol. 101, pp. 323-36 (2000)

GROUNDS OF REJECTION

Claim 3 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "an essentially homogeneous population of seed."

Claims 6 and 11 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "in accordance with."

Claims 15, 17 and 20 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "capable of expressing."

Claims 16 and 27-30 stand rejected under 35 U.S.C. § 112, second paragraph as failing to limit the scope of the claims from which they depend.

Claim 28 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of "the article 'a' in the recitation 'wherein the single locus was stably inserted into a corn genome.'"

Claim 29 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of a "locus ... consisting of a dominant allele and a recessive allele."

Claim 30 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrases "yield enhancement," "improved nutritional quality," and "enhanced yield stability."

Claims 16 and 24-31 stand rejected under the written description provision of 35 U.S.C. § 112, first paragraph.

Claims 16 and 24-31 stand rejected under the enablement provision of 35 U.S.C. § 112, first paragraph.

We reverse.

BACKGROUND

The present "invention relates to inbred corn seed and plants of the variety designated I180580, and derivatives and tissue cultures thereof." Specification, page 1. According to appellant (specification, page 26), "[a] description of the physiological and morphological characteristics of corn plant I180580 is presented in Table 3" of the specification, pages 26-27. On this record the examiner has indicated that claims drawn to plants, plant parts, and seed of the corn variety designated I180580 are allowable. See e.g., claims 1, 2,

5, 7-10, 12 and 13, and Answer, page 2, wherein the examiner states "[c]laims 1, 2, 5, 7-10, 12 [and] 13 ... stand allowed."

A second aspect of the present invention comprises hybrid plants and processes "for producing [first generation (F_1) hybrid[5]] corn seeds or plants, which ... generally comprise crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is a plant of the variety designated I180580." Specification, pages 7-9. On this record the examiner has indicated that claims drawn to a process of producing corn seed wherein the process comprises crossing a first parent corn plant with a second parent corn plant are allowable. See e.g., claims 21-23 and Answer, page 2, wherein the examiner states claims "21-23 stand allowed."

A third aspect of the present invention comprises single locus converted plants of the corn variety I180580. Specification, page 6. As appellant explains (specification, page 23, emphasis added), single locus converted (conversion) plants are those plants

> which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. A single locus may comprise one gene, or in the case of transgenic plants,

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[5] According to the specification (page 20), a F_1 hybrid is "[t]he first generation progeny of the cross of two plants." Accordingly, we understand claims 24 and 25 to refer to F_1 hybrids. In this regard, we note that similar claims, directed to a different corn variety, were presented for our review in Appeal Nos. 2004-1506 and 2004-2317. During the oral hearing in Appeal Nos. 2004-1506 and 2004-2317, appellant's representative confirmed that all claims drawn to hybrid plants or hybrid seeds (see e.g., claims 24 and 25) refer to F_1 hybrids. Further, based on our understanding of claim 25, claim 26 before us on appeal fails to further limit claim 25 from which it depends. See "Other Issues," infra.

> one or more transgenes integrated into the host genome at a single site (locus).

As appellant explains (specification, bridging paragraph, pages 29-30):

> Many single locus traits have been identified that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques. Single locus traits may or may not be transgenic; examples of these traits include, but are not limited to, male sterility, waxy starch, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, male fertility, enhanced nutritional quality, industrial usage, yield stability, and yield enhancement. These genes are generally inherited through the nucleus, but may be inherited through the cytoplasm. Some known exceptions to this are genes for male sterility, some of which are inherited cytoplasmically, but still act as single locus traits.

A final aspect of the present invention is directed to a process of producing an inbred corn plant derived from a plant of the corn variety I180580. See e.g., claim 31. According to appellant's specification (page 10),

> the present invention provides a method of producing an inbred corn plant derived from the corn variety I180580, the method comprising the steps of: (a) preparing a progeny plant derived from corn variety I180580, wherein said preparing comprises crossing a plant of the corn variety I180580 with a second corn plant, and wherein a sample of the seed of corn variety I180580 has been deposited under ATCC Accession No. ... [PTA-4490]; (b) crossing the progeny plant with itself or a second plant to produce a seed of a progeny plant of a subsequent generation; (c) growing a progeny plant of a subsequent generation from said seed of a progeny plant of a subsequent generation and crossing the progeny plant of a subsequent generation with itself or a second plant; and (d) repeating steps (c) and (d) for an addition 3-10 generations to produce an inbred corn plant derived from the corn variety I180580. In the method, it may be desirable to select particular plants resulting from step (c) for continued crossing according to steps (b) and (c). By selecting plants having one or more desirable traits, an inbred corn plant derived from the corn variety I180580 is obtained which possesses some of the desirable traits of corn variety I180580 as well potentially other selected traits.

Therefore, as we understand this aspect of the claimed invention (e.g., claim 31), the claim is drawn to a process wherein an inbred corn plant is derived from the corn variety I180580.

As appellant explains (specification, page 3),

> The development of uniform corn plant hybrids requires the development of homozygous inbred plants, the crossing of these inbred plants, and the evaluation of the crosses. Pedigree breeding and recurrent selection are examples of breeding methods used to develop inbred plants from breeding populations. Those breeding methods combine the genetic backgrounds from two or more inbred plants or various other broad-based sources into breeding pools from which new inbred plants are developed by selfing and selection of desired phenotypes. The new inbreds are crossed with other inbred plants and the hybrids from these crosses are evaluated to determine which of those have commercial potential.

We emphasize, that while "new inbreds" having commercial potential may result from the method set forth in claim 31, the claim does not encompass any specific plant that is produced as a result of the method. Rather the claim encompasses only a method of producing an inbred corn plant that is "derived" from the corn variety I180580. The examiner has indicated that a claim drawn to a corn plant of the corn variety I180580 is allowable. See e.g., claim 5, and Answer, page 2, wherein the examiner states that claim 5 is allowed.

Against this backdrop, we now consider the rejections of record.

DISCUSSION

Definiteness:

Claims 3, 6, 11, 15-20 and 27-30 stand rejected under 35 U.S.C. § 112, second paragraph. For the following reasons we reverse.

Claim 3

Claim 3 depends from independent claim 2, and stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "an essentially homogeneous population of seed...." Answer, page 3. According to the examiner (Answer, page 5), claim 2 is drawn "to a single variety of seed, that of corn variety I180580." Thus, the examiner finds (Answer, page 4), the population of seed set forth in claim 2 "is a homogeneous population of genetically inbred seed." Accordingly, the examiner finds (id.), "the 'essentially homogeneous' language [in claim 3] ... appear[s] to be superfluous."

However, as disclosed in appellant's specification (page 5),

> [e]ssentially homogeneous populations of inbred seed are those that consist essentially of the particular inbred seed, and are generally free from substantial numbers of other seed, so that the inbred seed forms between about 90% and about 100% of the total seed, and preferably, between about 95% and about 100% of the total seed.

Accordingly, we disagree with the examiner's assertion (Answer, page 5) that claim 3 is unclear simply because it may contain seed other than the seed of the corn variety I180580. We remind the examiner that claim language must be analyzed "not in a vacuum, but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary skill in the pertinent art." In re Moore, 439 F.2d 1232, 1235, 169 USPQ 236, 238 (CCPA 1971). In our opinion a person of ordinary skill in the art would recognize that an essentially homogeneous population of seed of the corn variety I180580 is a population of seed that is generally free

from substantial numbers of other seed, e.g., wherein corn variety I180580 seed forms between about 90% and about 100% of the total seed in the population.[6]

We also note that this rejection appears to be inconsistent with the indication that claim 14 is allowable. Answer, page 2. Claim 14 is directed to "[a]n essentially homogeneous population of corn plants produced by growing the seed of the corn variety I180580...." Emphasis added. The examiner's logic as applied to claim 3 would appear to apply to claim 14 as well, yet claim 14 is indicated as allowable, while claim 3 stands rejected. Cf. claims 3 and 14 of Appeal Nos. 2004-1506 and 2004-2317, wherein claims similar to claims 3 and 14 were presented for our review. In Appeal Nos. 2004-1506 and 2004-2317 the examiner of record, rejected claims 3 and 14 under 35 U.S.C. 112, second paragraph, applying the same rationale as the examiner applied to claim 3 herein. Accordingly, not only has the examiner treated the claims on this record in an inconsistent manner, the examiner has treated the claims in a manner that is inconsistent with the prosecution of similar claims in other related applications.

Accordingly, we reverse the rejection of claim 3 under 35 U.S.C. § 112, second paragraph.

Claims 6 and 11

Claims 6 and 11 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "in accordance with." According to the

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[6] Cf. the examiner's statement (Answer, page 6), "[i]f claim 3 were amended to read --- [a]n essentially homogeneous population of corn seeds consisting essentially of the inbred corn seed of claim 1 ---, the claim would have a definite meaning."

examiner (Answer, page 9), it is unclear if a plant "that generally follows the trend of the profile of Table 6, but which differs at one or a few loci, [would] be considered in 'conformity' or 'in accordance' with the profile of Table 6."

On this record, we understand the phrase "in accordance with" as it is used in claims 6 and 11 to mean "the same"[7]. Stated differently, we understand the claims to read:

> 6. The corn plant of claim 5, having:
> (a) the same SSR profile as shown in Table 6; or
> (b) the same isozyme typing profile as shown in Table 7.
>
> 11. The plant part of claim 10, wherein said cell is further defined as having:
> (a) The same SSR profile as shown in Table 6; or
> (b) The same isozyme typing profile as shown in Table 7.

Accordingly we reverse the rejection of claims 6 and 11 under 35 U.S.C. § 112, second paragraph.

<u>Claims 15, 17 and 20</u>

Claims 15, and 17-20 stand rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrase "capable of expressing," or "capable of regenerating." According to the examiner (Answer, page 10), the claims do "not make clear if the plant actually expresses the traits, or when or under what conditions the traits are expressed." In this regard, the examiner finds (Answer, bridging paragraph, pages 10-11),

> while the plant has the capacity to express the characteristics, for some reason it may not. Certain characteristics of a plant are

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[7] Cf. Appeal Nos. 2004-1506 and 2004-2317, which use similar language for claims directed to different corn varieties. In this regard, we note that during the February 10, 2005 oral hearing in Appeal Nos. 2004-1506 and 2004-2317, appellant's representative confirmed that the phrase "in accordance with" was intended to mean "the same".

> expressed only at certain times of its life cycle, and are incapable of being expressed at other times. The colors of flower parts such as silks, or fruit parts such as husks, are examples. The promoters of many genes conferring traits require a transcription factor to become active. Is a plant that has such a gene, but not the transcription factor, considered "capable of expressing" that gene, and the trait associated with that gene, and is such a plant encompassed by the claims?

To address the examiner's concerns, we find it sufficient to state that if a plant has the capacity to express the claimed characteristics it meets the requirement of the claim regarding "capable of," notwithstanding that due to a particular phase of the life cycle the plant is not currently expressing a particular characteristic. Alternatively, if a plant is incapable of expressing the claimed characteristics at any phase of the life cycle, because it lacks, for example, the "transcription factor" required for expression – such a plant would not meet the requirement of the claim regarding "capable of."

Here, we find the examiner's extremely technical criticism to be a departure from the legally correct standard of considering the claimed invention from the perspective of one possessing ordinary skill in the art.[8] In our opinion, a person of ordinary skill in the art would understand what is claimed. Amgen Inc. v. Chugai Pharmaceutical Co., Ltd., 927 F.2d 1200, 1217, 18 USPQ2d 1016, 1030 (Fed. Cir. 1991). We find the same to be true for the phrase "capable of" as set forth in claims 17 and 20.

Accordingly we reverse the rejection of claims 15, 17 and 20 under 35 U.S.C. § 112, second paragraph.

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[8] Cf. Digital Equipment Corp. v. Diamond, 653 F.2d 701, 724, 210 USPQ 521, 546 (CA 1981).

Claims 16 and 27-30

Claims 16 and 27-30 stand rejected under 35 U.S.C. § 112, second paragraph as failing to limit the scope of the claims from which they depend. According to the examiner (Answer, page 8), since the plant set forth in claim 16 is male sterile it cannot express all the morphological and physiological characteristics of the male fertile corn variety I180580. Similarly, the examiner finds it unclear whether the plant set forth in claim 27 has all the characteristics of the plant set forth in claim 5, from which claim 27 depends. Answer, pages 9-10. In response, appellant asserts (Brief, page 8), claims 16 and 27 simply add a further limitation to the claims from which they depend. We agree.

For example, claim 16 reads on a corn plant capable of expressing all the physiological and morphological characteristics of the corn variety I180580, further comprising a nuclear or cytoplasmic gene conferring male sterility. In our opinion, the claims reasonably apprise those of skill in the art of their scope. Amgen, As set forth in Shatterproof Glass Corp. v. Libbey-Owens Ford Co., 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir. 1985), "[i]f the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more."

Accordingly we reverse the rejection of claims 16 and 27-30 under 35 U.S.C. § 112, second paragraph.

Claim 28

Claim 28 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of "the article 'a' in the recitation 'wherein the single locus was stably inserted into a corn genome.'" According to the examiner (Answer, page 13), "the recitation does not make clear if the genome is that of I180580 or that of a different corn plant."

According to appellant's specification (page 22, emphasis removed), a "Single Locus Converted (Conversion) Plant" refers to

> [p]lants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. A single locus may comprise one gene, or in the case of transgenic plants, one or more transgenes integrated into the host genome at a single site (locus).

Accordingly, we agree with appellant (Brief, page 9) "[t]he single locus referred to in claim 28 may or may not have been directly inserted into the genome of the claimed plant." As we understand the claim, and arguments of record, claim 28 presents two possibilities: (1) the single locus is directly inserted into the claimed plant and nothing further need be done; or (2) the single locus is directly inserted into a different plant, which is then used to transfer the single locus to the claimed plant through use of the plant breeding technique known as backcrossing.

In our opinion, the claim reasonably apprises those of skill in the art of its scope.[9] Amgen. Accordingly, we reverse the rejection of claim 28 under 35 U.S.C. § 112, second paragraph.

Claim 29

The examiner finds (Answer, page 11), claim 29 is indefinite in the recitation of a locus selected from the group consisting of a dominant allel and a recessive allele. According to the examiner (id.), "a locus is a location on a genome, and is not an allele." As appellant explains (Brief, page 9), page 22 of the specification discloses that "[a] single locus may comprise one gene, or in the case of transgenic plants, one or more transgenes integrated into the host genome at a single site (locus)." According to appellant (id.), "a single locus conversion refers to the gene introduced and not a locus on the chromosome. [Thus,] [t]here is ... nothing indefinite in the recitation of a single locus conversion that is a dominant allele or a recessive allele."

In response, the examiner finds (Answer, page 12), "[i]f claim 29 read, --the conversion—rather than 'the locus', [a]ppellant's arguments would be more persuasive." Claim 29 depends from claim 27, which is drawn to "[t]he corn plant of claim 5, further defined as having a genome comprising a

[9] The same is true of the examiner's comment (Answer, bridging sentence, pages 10-11) that the claim is confusing since "a locus is a position on a genome rather than a piece of DNA or a gene." As appellant explains (specification, page 22), "[a] single locus may comprise one gene, or in the case of transgenic plants, one or more transgenes integrated into the host genome at a single site (locus)."

single locus conversion." In claim 29, appellant chose to refer to this "single locus conversion" as "the locus." As we understand the rejection, the examiner prefers that appellant refer to the "single locus conversion" of claim 27 as "the conversion." We fail to see why referring to the "single locus conversion" of claim 27 as the "conversion" would be any clearer than appellant's use of the term "locus."[10] In our opinion, the claim reasonably apprises those of skill in the art of its scope. Amgen. Accordingly, we reverse the rejection of claim 29 under 35 U.S.C. § 112, second paragraph.

Claim 30

Claim 30 stands rejected under 35 U.S.C. § 112, second paragraph as indefinite in the recitation of the phrases "yield enhancement," "improved nutritional quality," and "enhanced yield stability." According to the examiner the terms "yield enhancement," "improved nutritional quality," and "enhanced yield stability" are relative and have no definite meaning. Answer, pages 12-13. The examiner is correct (id.), when a word of degree is used appellant's specification must provide some standard for measuring that degree. Seattle Box. Co. v. Industrial Crating & Packing, Inc., 731 F.2d 818, 826, 221 USPQ 568, 573-574 (Fed. Cir. 1984).

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[10] To this end, we note that a similar claim was presented for our review in Appeal Nos. 2004-1506 and 2004-2317. See claim 29 presented for our review in each appeal. However, the examiner of record in Appeal Nos. 2004-1506 and 2004-2317 apparently found that a person of ordinary skill in the art would have understood the scope of the claim, as no comment was made on either of these records, to reflect that the language of claim 29 was indefinite with regard to appellant's use of the term "locus" in referring to "a single locus conversion," as is presented herein for our review.

On this record, appellant asserts (Brief, page 10), it is "understood the enhancement of yield or yield stability and improved nutritional quality is relative to a plant lacking the single locus. The metes and bounds of the claim are thus fully understood by one of skill in the art and the use of the terms is not indefinite." On reflection, we agree with appellant. The fact that some claim language is not mathematically precise does not per se render the claim indefinite. Seattle Box. As set forth in Shatterproof Glass, "[i]f the claims, read in the light of the specifications, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more." In our opinion, a person of ordinary skill in the art would have understood the enhancement of yield or yield stability and improved nutritional quality is relative to a plant lacking the single locus.

Accordingly we reverse the rejection of claim 30 under 35 U.S.C. § 112, second paragraph.

Written Description:

Claims 16, and 24-31 stand rejected under 35 U.S.C. § 112, first paragraph, as the specification fails to adequately describe the claimed invention. For the following reasons, we reverse.

Claims 24-26

Claims 24-26 depend from claim 23. On this record, the examiner has indicated that claim 23 is allowable. Answer, page 2. The examiner finds

(Answer, page 14), claims 24-26 are drawn to a hybrid plant or seed "produced by crossing inbred corn plant I180580 with any second, distinct inbred corn plant."

As we understand it, based on this construction of claims 24-26, the examiner is of the opinion that since the hybrids inherit only ½ of their diploid[11] set of chromosomes from the plant of corn variety I180580, a person of skill in the art would not have viewed the teachings of the specification as sufficient to demonstrate that appellant was in possession of the genus of hybrid seeds and plants encompassed by claims 24-26. According to the examiner (Answer, bridging sentence, pages 28-29), "[t]he fact that any hybrid plant will inherit half of its alleles from I180580 then does not provide sufficient description of the morphological and physiological characteristics expressed by the claimed hybrid plants."

There is no doubt that the expressed gene products of a hybrid plant, e.g., the morphological and physiological traits, of I180580 and a non-I180580 corn plant will depend on the combination of the genetic material inherited from both parents. See Answer, page 28. Nevertheless, we disagree with the examiner's conclusion (Answer, bridging sentence, pages 28-29) that "[t]he fact that any hybrid plant will inherit half of its alleles from I180580 then does not provide sufficient description of the morphological and physiological characteristics expressed by the claimed hybrid plants."

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[11] According to appellant's specification (page 20), diploid means "a cell or organism having two sets of chromosomes."

On these facts, we find it necessary to take a step back and consider what is claimed. The claims are drawn to a F_1 hybrid seed (claim 24) or plant (claim 25) resulting from a cross between a plant of corn variety I180580 and a non-I180580 corn variety. The claims do not require the hybrid to express any particular morphological or physiological characteristic. Nor do the claims require that a particular non-I180580 corn variety be used.[12] All that is required by the claims is that the hybrid has one parent that is a plant of corn variety I180580. Since the examiner has indicated that the seed and the plant of the corn variety I180580 are allowable (see claims 1 and 5), there can be no doubt that the specification provides an adequate written description of this corn variety. In addition, the examiner appears to recognize (Answer, page 14) that appellant's specification describes an exemplary hybrid wherein one parent was a plant of the corn variety I180580, see e.g., specification, pages 52-56. Accordingly, it is unclear to this merits panel what additional description is necessary.

As set forth in Reiffin v. Microsoft Corp., 214 F.3d 1342, 1345, 54 USPQ2d 1915, 1917 (Fed. Cir. 2000), the purpose of the written description requirement is to "ensure that the scope of the right to exclude, as set forth in the claims does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification." Here the hybrid seed or plant has

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[12] According to appellant (Brief, page 18), "hundreds or even thousands of different inbred corn lines were well known to those of skill in the art prior to the filing [date] of the instant application, each of which could be crossed to make a hybrid plant with in the scope of the claims."

one parent that is a plant of the corn variety I180580. To that end, to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). For the foregoing reasons it is our opinion that appellant has provided an adequate written description of the subject matter set forth in claims 24-26.

We recognize the examiner's argument relating to molecular markers (Answer, pages 31-32), as well as the examiner's arguments concerning a correlation between the hybrid's genome structure and the function of the hybrid plant (e.g., Answer, page 30). However, for the foregoing reasons, we are not persuaded by these arguments.

Claims 16 and 27-30

According to the examiner (Answer, page 17), "the specification provides no description of any plant produced by classical breeding methods such as backcrossing, as claimed in claims 16 and 27-31." In this regard, the examiner finds (Answer, bridging paragraph, pages 17-18),

> the individual genes conferring the desired traits (to be introgressed via classical breeding methods such as backcrossing) have not been characterized with respect to either sequence or source organism, and the genes for several of the contemplated traits; i.e. "improved nutritional quality", "yield enhancement" and "enhanced yield stability" as recited in claim 30, or "industrial usage" as recited on page 29 of the specification, lines 27-28; have not been isolated either by [a]ppellant or by the skilled artisan. In fact, the genes conferring such traits are thought to be quantitative in nature, i.e. governed by multiple genes, often occurring on different chromosomes, which additively contribute to the desired effect.

More specifically, the examiner finds (Answer, page 22), claims 27-29 "broadly encompass single loci that have not been discovered or isolated." To the extent that the examiner is asserting that appellant has not provided an enabling disclosure of single loci that have not been identified, we note that to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath.

To the extent that the examiner is of the opinion (Answer, bridging paragraph, pages 17-18) that single genes that alone govern "yield enhancement" or "enhanced yield stability" have not been discovered, the examiner provides no evidence to support such an assertion, or that "genes conferring such traits are though to be quantitative in nature...." In this regard, we note that appellant discloses (specification, page 29), "[m]any single locus traits have been identified ... examples of these traits include, but are not limited to, ... enhanced nutritional quality, industrial usage, yield stability, and yield enhancement." It appears that the examiner has overlooked appellant's assertion that single locus traits for yield stability and yield enhancement are well known in the art. To this end, we direct the examiner's attention to, for example, United States Patent No. 5,936,145 ('145)[13], issued August 10, 1999, which is

[13] We note that the assignee of the '145 patent is DeKalb Genetics Corporation. The assignee of the present application is Monsanto Company, the parent of wholly-owned subsidiary DeKalb Genetics Corporation.

prior to the filing date of the instant application. For clarity, we reproduce claims 8, 29 and 39 of the '145 patent below:

> 8. A corn plant having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.
>
> 29. The corn plant of claim 8, further comprising a single gene conversion.
>
> 39. The single gene conversion of the corn plant of claim 29, where the gene confers enhanced yield stability.

As we understand it, claim 39 of the '145 patent, is drawn to a corn plant which comprises a single gene conversion, wherein the gene confers enhanced yield stability. Thus, contrary to the examiner's assertion it appears, for example, that a single gene that confers enhanced yield stability was known in the art prior to the filing date of the instant application. We remind the examiner "a patent need not teach, and preferably omits, what is well known in the art." Hybritech Incorporated v. Monoclonal Antibodies, Inc. 802 F.2d 1367, 1385, 231 USPQ 81, 94 (Fed. Cir. 1986).

We remind the examiner that the inquiry into whether the description requirement is met must be determined on a case-by-case basis and is a question of fact. In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). A description as filed is presumed to be adequate; unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption. See e.g., In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description.

Accordingly, it is the examiner who has the initial burden of establishing by a preponderance of evidence that a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. Wertheim, 541 F.2d at 263, 191 USPQ at 97. On this record, the examiner provides no evidence to support his assertions.

Furthermore, we recognize the examiner's assertion (Answer, page 23) "that claim 16 as written is drawn to a corn plant which simultaneously is male sterile and male fertile, as discussed in the rejection under 35 U[.]S[.]C[. §] 112, second paragraph." We disagree with the examiner's construction of the claim 16. As we understand it, claim 16 is drawn to a corn plant capable of expressing all the physiological and morphological characteristics of the corn variety I180580 further comprising a nuclear or cytoplasmic gene conferring male sterility. In contrast to the examiner's construction of claim 16, the claim is drawn to a plant that of the corn variety I180580 that is male sterile. In this regard, we direct the examiner's attention to claim 16 of related Appeal Nos. 2005-1506 and 2004-2317, which differ from claim 16 on this record only in the variety of corn. In addition, we note that the disclosure of Appeal Nos. 2005-1506 and 2004-2317 and the instant application are substantially similar. However, in both Appeal Nos. 2005-1506 and 2004-2317 the examiner apparently found that appellant's specification provided an adequate written description of claim 16 as no rejection of this claim was made under the written description provision of 35 U.S.C. § 112, first paragraph. Accordingly, we find that the examiner has treated claim 16 in a manner that is inconsistent with the prosecution of similar claims in

related applications 09/788,334 and 09/771938, which is the subject matter of Appeal Nos. 2004-1506 and 2004-2317 respectively.

For the foregoing reasons, we are not persuaded by the examiner's arguments.

Claim 31

Claim 31 is drawn to a method of producing an inbred corn plant derived from the corn variety I180580. The claimed method begins by crossing a plant of the corn variety I180580 with any other corn plant. The method requires that the progeny corn plant be crossed either to itself, or with any other corn plant, and that the progeny of this cross be further crossed to itself, or with another corn plant, and so on throughout several generations. As we understand it, claim 31, in its simplest form, is directed to a method of using a plant of the corn variety I180580 to produce an inbred corn plant.

Nevertheless, the examiner finds (Answer, page 20),

> [t]he specification fails to disclose or describe any progeny resulting from such crosses, wherein said progeny could contain only a small portion of the I180580 genome, it any at all, and wherein said progeny would contain a majority of undisclosed and uncharacterized genetic material from a multitude of undisclosed and uncharacterized parents. Furthermore, no description has been provided for the progeny of such crosses with regard to even one morphological trait of said progeny containing a majority of non-I180580 genetic material.

As we understand the examiner's argument, not only does appellant have to provide a written description of the starting corn plant (I180580), but appellant also must look into the future to determine every other potential corn plant that someone may wish to cross with the I180580 corn variety, and provide written

descriptive support for not only every other corn plant that could be crossed with I180580, but also the resulting progeny of each cross.

As set forth in Reiffin, the purpose of the written description requirement is to "ensure that the scope of the right to exclude, as set forth in the claims does not overreach the scope of the inventor's contribution to the field of art as described in the patent specification." Here the method of producing an inbred corn plant requires a plant of the corn variety I180580 be used as the starting material. To that end, to satisfy the written description requirement, the inventor "must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention" [emphasis added]. Vas-Cath. The examiner has indicated that a claim to a plant of the corn variety I180580 is allowable, see e.g., appellant's claim 5. Therefore, in our opinion, there can be no doubt that appellant was in possession of a plant of the corn variety I180580, in addition to a method of using that plant to cross with any other corn plant to produce an inbred corn plant as set forth in appellant's claim 31.

In our opinion, it matters not what the other corn plants are, or what the progeny of a cross between corn variety I180580 and some other corn plant represents. The invention of claim 31 is drawn to the use of the corn variety I180580 as the starting material to produce an inbred corn plant. Accordingly, for the foregoing reasons, it is our opinion that appellant has "convey[ed] with reasonable clarity to those skilled in the art that, as of the filing date sought, [they were] in possession of the invention," Vas-Cath (emphasis omitted).

Summary

For the foregoing reasons, we reverse the rejection of claims 16, 24-31 under the written description provision of 35 U.S.C. § 112, first paragraph.

Enablement:

Claims 16 and 24-31 stand rejected under the enablement provision of 35 U.S.C. § 112, first paragraph. The examiner finds (Answer, page 36), claims 16 and 24-31 "are broadly drawn to corn plants containing a multitude of exemplified or non-exemplified single gene conversions or transgenes of any sequence and from any source organism" (claims 16 and 27-30); to "any hybrid corn seed produced by the process of crossing the inbred corn plant I180580 with any second, distinct, inbred corn plant, and any hybrid corn plant produced by growing said hybrid corn seed" (claims 24-26); to methods of repeatedly crossing I180580 with any other undefined non-I180580 parent over multiple generations" (claim 30); and to a plant (claim 16) that is simultaneously male fertile and male sterile. The examiner presents several lines of argument under this heading. We take each in turn.

I. A plant that is simultaneously male fertile and male sterile:

According to the examiner (Answer, page 37), "no guidance has been provided for how to make an I180580 corn plant which is simultaneously male fertile and male sterile, as claimed in claim 16. As discussed supra, we disagree with the examiner's construction of the claim 16. As we understand it, claim 16 is drawn to a corn plant capable of expressing all the physiological and

morphological characteristics of the corn variety I180580 further comprising a nuclear or cytoplasmic gene conferring male sterility. In contrast to the examiner's construction of claim 16, the claim is drawn to a plant of the corn variety I180580 that is male sterile. The examiner has provided no evidence on this record that would suggest that such a plant could not be made. Accordingly, we are not persuaded by the examiner's argument.

In this regard, we direct the examiner's attention to claim 16 of related Appeal Nos. 2005-1506 and 2004-2317, which differ from claim 16 on this record only in the variety of corn. In addition, we note that the disclosure of Appeal Nos. 2005-1506 and 2004-2317 and the instant application are substantially similar. However, in both Appeal Nos. 2005-1506 and 2004-2317 the examiner apparently found that appellant's specification provided an enabling disclosure of claim 16 as no rejection of this claim was made under the enablement provision of 35 U.S.C. § 112, first paragraph. Accordingly, we find that the examiner has treated claim 16 in a manner that is inconsistent with the prosecution of similar claims in related applications 09/788,334 and 09/771938, which is the subject matter of Appeal Nos. 2004-1506 and 2004-2317 respectively.

II. <u>Retaining all the genetic and morphological fidelity of I180580</u>:

According to the examiner (Answer, page 40), "[i]t is not clear that single loci may be introduced into the genetic background of plant through traditional breeding, while otherwise maintaining the genetic and morphological fidelity of the original inbred variety...." More specifically, the examiner finds (Answer,

page 37), "no guidance has been provided for preventing the introduction of unwanted genetic material conferring undesirable agronomic traits from the donor breeding partner to I180580." With reference to Hunsperger, Kraft, and Eshed the examiner asserts (Answer, page 47), "[t]he rejection raises the issue of how linkage drag hampers the insertion of single genes alone into a plant by backcrossing, while recovering <u>all</u> of the original plant's genome."

We note, however, that claims 24-31 do not require that the hybrid, or single locus conversion plant <u>retain all of the morphological and physiological traits</u> of the parent plant in addition to exhibiting the single trait conferred by the introduction of the single loci. Nor do claims 27-30 require that the resultant plant retain all of the original plant's <u>genome</u> in addition to the single locus transferred into the inbred <u>via</u> the backcrossing technique.

As appellant explains (specification, page 28, emphasis added),

> [t]he term single locus converted plant as used herein refers to those corn plants which are developed by a plant breeding technique called backcrossing wherein <u>essentially all</u> of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single locus transferred into the inbred via the backcrossing technique.

<u>See also</u> appellant's definition of single locus converted (conversion) plant at page 22 of the specification. We find nothing in the appellant's specification to indicate that the single locus converted plant retains <u>all</u> of the morphological and physiological traits, or <u>all</u> of the genome, of the parent plant in addition to the single locus transferred <u>via</u> the backcrossing technique. Accordingly, we disagree with the examiner's assertions to the contrary.

Further, as discussed supra, claim 16 is drawn to a corn plant capable of expressing all the physiological and morphological characteristics of the corn variety I180580, further comprising a nuclear or cytoplasmic gene conferring male sterility. In this regard, we note that appellant's specification discloses (page 31), "[e]xamples of male-sterility genes and corresponding restorers which could be employed with the inbred of the invention as well known to those of skill in the art of plant breeding and are disclosed in, for instance, U.S. Patent No. 5,530,191; U.S. Patent No. 5,689,041; U.S. Patent No. 5,741,684; and U.S. Patent No. 5,684,242...." We find no evidence in the Answer to suggest this disclosure in appellant's specification is incorrect, or insufficient. In addition, we note that the examiner's rejection of claim 16 is inconsistent with the manner in which a similar claim was treated in related applications 09/788,334 and 09/771,938, the subject matter of Appeal Nos. 2004-1506 and 2004-2317 respectively. Claim 16 of related applications 09/788,334 and 09/771,938, differs from claim 16 of the instant application only with regard to the corn variety. Nevertheless, while the disclosure in these related applications is substantially similar to the disclosure of the instant application, claim 16 was not rejected under the enablement provision of 35 U.S.C. § 112, first paragraph, in either of related applications 09/788,334 or 09/771,938.

Further, we recognize appellant's argument (Brief, page 25) that the examiner failed to establish a nexus between Hunsperger's discussion of petunias; Kraft's discussion of sugar beets; and Eshed's discussion of tomatoes, and the subject matter of the instant application - corn. Absent evidence to the

contrary, we agree with appellant (Reply Brief, page 10), "[t]he [examiner's] indication[14] that the references concerning petunias, sugar beets and tomatoes apply to corn is made without any support." That the examiner has failed to identify (Answer, page 47) an example "in the prior art of plants in which linkage drag does not occur," does not mean that linkage drag is expected to occur in corn breeding, which according to appellant (Reply Brief, page 9) "is extremely advanced and well known in the art...." In this regard, we agree with appellant (Brief, pages 21-22; Accord Reply Brief, page 10), the examiner has improperly placed the burden on appellant to demonstrate that the examiner's unsupported assertion is not true. We remind the examiner, as set forth in In re Wright, 999 F.2d 1557, 1561-62, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993):

> When rejecting a claim under the enablement requirement of section 112, the PTO bears an initial burden of setting forth a reasonable explanation as to why it believes that the scope of protection provided by that claim is not adequately enabled by the description of the invention provided in the specification of the application; this includes, of course, providing sufficient reasons for doubting any assertions in the specification as to the scope of enablement.

III. The single locus to be introduced:

The examiner finds (Answer, page 44), "the claims do not place any limit on the single locus conversions or transgenes to be introduced" into I180580 plants. The examiner recognizes, however, that "[t]he prior art shows that hundreds of nucleotide sequences encoding products that confer various types

[14] See Answer page 47, wherein the examiner asserts "[l]inkage drag appears to be a phenomenon that occurs in all plant types."

of plant traits have been isolated at the time the instant invention was filed." Id. In addition, the examiner recognizes (id.), "[o]ne skilled in the art can transform any of these isolated nucleotide sequences known in the prior art into a corn plant cell, and regenerate a transgenic plant from the transformed cell."

Nevertheless, the examiner finds (Answer, page 45), "[u]ndue experimentation would be required by one skilled to make and use the claimed invention." In this regard, the examiner asserts (Answer, page 44) that the claims broadly encompass corn plants comprising any type of single loci, including those that have not yet to be isolated. To the extent that the examiner is asserting that appellant has not provided an enabling disclosure of single loci that have not been identified, we note that enablement under 35 U.S.C. § 112, first paragraph is evaluated as of appellant's filing date. As set forth in Chiron Corp. v. Genentech Inc., 363 F.3d 1247, 1254, 70 USPQ2d 1321, 1325-26 (Fed. Cir. 2004), "a patent document cannot enable technology that arises after the date of application. The law does not expect an applicant to disclose knowledge invented or developed after the filing date. Such disclosure would be impossible. See In re Hogan, 559 F.2d 595, 605-06 [194 USPQ 527] (CCPA 1977)."

The examiner's comment, however, may be directed to his assertion (Answer, page 44) that "isolated genes whose products confer yield enhancement, enhanced yield stability, improved nutritional content, or industrial usage, are not known in the prior art." However, as discussed, supra, it appears that contrary to the examiner's assertion a single locus that confers the trait of, for example, yield enhancement was known in the art prior to the filing date of

the instant invention. In addition, as discussed, supra, appellant's specification asserts that such traits were known in the art. See specification, page 31. Accordingly, as set forth in In re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971), the burden is on

> the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure.

On this record, we find only the examiner's unsupported conclusions as to why the specification does not enable the claimed invention. We remind the examiner that nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples. Marzocchi, 439 F.2d at 223, 169 USPQ at 369. In the absence of an evidentiary basis to support the rejection, the examiner has not sustained his initial burden of establishing a prima facie case of non-enablement. In this regard, we note that the burden of proof does not shift to appellant until the examiner first meets his burden. Marzocchi, 439 F.2d at 223-224, 169 USPQ at 369-370.

We also recognize the examiner's assertion (Answer, bridging paragraph, pages 44-45) that "transgene or single locus conversion DNA, as broadly interpreted, includes isolated genes whose functions are not known ... [or where] the effects of transgene or single locus conversion expression on the traits expressed by untransformed or unconverted I180580 are unknown." While this

may be true, the examiner has not provided any evidence to suggest that it would require undue experimentation to obtain a single locus converted plant wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the characteristics conferred by the single locus transferred into the inbred via the backcrossing technique. See specification, bridging paragraph, pages 28-29.

While it is not expressly stated in the text of the examiner's rejection, it may be that the examiner is concerned that the claims include inoperative embodiments. If so, the examiner is directed to Atlas Powder Co. v. E.I. DuPont De Nemours & Co., 750 F.2d 1569, 1576-77, 224 USPQ 409, 414 (Fed. Cir. 1984):

> Even if some of the claimed combinations were inoperative, the claims are not necessarily invalid. "It is not a function of the claims to specifically exclude ... possible inoperative substances...." In re Dinh-Nguyen, 492 F.2d 856, 859-59, 181 USPQ 46, 48 (CCPA 1974)(emphasis omitted). Accord, In re Geerdes, 491 F.2d 1260, 1265, 180 USPQ 789, 793 (CCPA 1974); In re Anderson, 471 F.2d 1237, 1242, 176 USPQ 331, 334-35 (CCPA 1971). Of course, if the number of inoperative combinations becomes significant, and in effect forces one of ordinary skill in the art to experiment unduly in order to practice the claimed invention, the claims might indeed be invalid. *See* e.g., In re Cook, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971).

On this record, the examiner provides no evidence that the number of inoperative embodiments is so large that a person of ordinary skill in the art would have to experiment unduly to practice the claimed invention. To the contrary, the examiner recognizes (Answer, page 44) that "[t]he prior art shows that hundreds of nucleotide sequences encoding products that confer various

types of plant traits have been isolated at the time the instant invention was filed"; and that "[o]ne skilled in the art can transform any of these isolated nucleotide sequences known in the prior art into a corn plant cell, and regenerate a transgenic plant from the transformed cell." Accordingly, we are not persuaded by the examiner's unsupported assertions.

For the foregoing reasons, we reverse the rejection of claims 27-30 under the enablement provision of 35 U.S.C. § 112, first paragraph.

OTHER ISSUES

As discussed supra, n. 5, we understand claims 24 and 25 to refer to F_1 hybrids. In this regard, we note that similar claims, directed to a different corn variety, were presented for our review in Appeal Nos. 2004-1506 and 2004-2317. During the oral hearing in Appeal Nos. 2004-1506 and 2004-2317, appellant's representative confirmed that all claims drawn to hybrid plants or hybrid seeds (see e.g., claims 24 and 25) refer to F_1 hybrids. Further, based on our understanding of claim 25, claim 26 before us on appeal fails to further limit claim 25 from which it depends. Accordingly, prior to any further action on the merits, we encourage the examiner and appellant to work together to resolve this issue.

SUMMARY

We reverse the rejection of claims 3, 6, 11, 15-20, and 27-30 under 35 U.S.C. § 112, second paragraph.

We reverse the rejection of claims 16, and 24-31 under the written description provision of 35 U.S.C. § 112, first paragraph.

We reverse the rejection of claims 16, and 24-31 under the enablement provision of 35 U.S.C. § 112, first paragraph.

As noted in the "Other Issues," claim 26 does not appear to further limit the claim from which it depends.

REVERSED

Toni R. Scheiner
Administrative Patent Judge

Donald E. Adams
Administrative Patent Judge

Lora M. Green
Administrative Patent Judge

BOARD OF PATENT APPEALS AND INTERFERENCES

Robert E. Hanson
FULBRIGHT & JAWORSKI L.L.P.
A REGISTERED LIMITED LIABILITY PARTNERSHIP
600 CONGRESS AVENUE, SUITE 2400
AUSTIN, TX 78701

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	Examiner BPAI	Art Unit	Page of

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-5,936,145	08-1999	Bradbury	
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

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*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
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	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
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*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

US005936145A

United States Patent [19]

[11] Patent Number: 5,936,145

[45] Date of Patent: Aug. 10, 1999

Bradbury

[54] INBRED CORN PLANT 87DIA4 AND SEEDS THEREOF

[75] Inventor: Peter J. Bradbury, Madison, Wis.

[73] Assignee: DeKalb Genetics Corporation, DeKalb, Ill.

[21] Appl. No.: 09/017,996

[22] Filed: Feb. 3, 1998

Related U.S. Application Data

[60] Provisional application No. 60/037,305, Feb. 5, 1997.

[51] Int. Cl.[6] A01H 5/00; A01H 4/00; A01H 1/00; C12N 5/04

[52] U.S. Cl. 800/320.1; 800/298; 800/275; 800/271; 800/301; 800/302; 800/303; 435/412; 435/424; 435/430; 435/430.1

[58] Field of Search 800/320.1, 298, 800/275, 271, 303, 274, 302; 435/172.3, 172.1, 412, 424, 430, 430.1

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(List continued on next page.)

Primary Examiner—Gary Benzion
Attorney, Agent, or Firm—Arnold, White & Durkee

[57] ABSTRACT

According to the invention, there is provided an inbred corn plant designated 87DIA4. This invention thus relates to the plants, seeds and tissue cultures of the inbred corn plant 87DIA4, and to methods for producing a corn plant produced by crossing the inbred plant 87DIA4 with itself or with another corn plant, such as another inbred. This invention further relates to corn seeds and plants produced by crossing the inbred plant 87DIA4 with another corn plant, such as another inbred, and to crosses with related species. This invention further relates to the inbred and hybrid genetic complements of the inbred corn plant 87DIA4, and also to the RFLP and genetic isozyme typing profiles of inbred corn plant 87DIA4.

39 Claims, No Drawings

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INBRED CORN PLANT 87DIA4 AND SEEDS THEREOF

The present application claims the priority of co-pending U.S. Provisional Patent Application Serial No. 60/037,305, filed Feb. 5, 1997, the entire disclosure of which is incorporated herein by reference without disclaimer.

BACKGROUND OF THE INVENTION

I. Technical Field of the Invention

The present invention relates to the field of corn breeding. In particular, the invention relates to the inbred corn seed and plant designated 87DIA4, and derivatives and tissue cultures of such inbred plant.

II. Description of the Background Art

The goal of field crop breeding is to combine various desirable traits in a single variety/hybrid. Such desirable traits include greater yield, better stalks, better roots, resistance to insecticides, herbicides, pests, and disease, tolerance to heat and drought, reduced time to crop maturity, better agronomic quality, and uniformity in germination times, stand establishment, growth rate, maturity, and fruit size.

Breeding techniques take advantage of a plant's method of pollination. There are two general methods of pollination: a plant self-pollinates if pollen from one flower is transferred to the same or another flower of the same plant. A plant cross-pollinates if pollen comes to it from a flower on a different plant.

Corn plants (*Zea mays L.*) can be bred by both self-pollination and cross-pollination. Both types of pollination involve the corn plant's flowers. Corn has separate male and female flowers on the same plant, located on the tassel and the ear, respectively. Natural pollination occurs in corn when wind blows pollen from the tassels to the silks that protrude from the tops of the ear shoot.

Plants that have been self-pollinated and selected for type over many generations become homozygous at almost all gene loci and produce a uniform population of true breeding progeny, a homozygous plant. A cross between two such homozygous plants produce a uniform population of hybrid plants that are heterozygous for many gene loci. Conversely, a cross of two plants each heterozygous at a number of gene loci produces a population of hybrid plants that differ genetically and are not uniform. The resulting non-uniformity makes performance unpredictable.

The development of uniform corn plant hybrids requires the development of homozygous inbred plants, the crossing of these inbred plants, and the evaluation of the crosses. Pedigree breeding and recurrent selection are examples of breeding methods used to develop inbred plants from breeding populations. Those breeding methods combine the genetic backgrounds from two or more inbred plants or various other broad-based sources into breeding pools from which new inbred plants are developed by selfing and selection of desired phenotypes. The new inbreds are crossed with other inbred plants and the hybrids from these crosses are evaluated to determine which of those have commercial potential.

The pedigree breeding method for single-gene traits involves crossing two genotypes. Each genotype can have one or more desirable characteristics lacking in the other; or, each genotype can complement the other. If the two original parental genotypes do not provide all of the desired characteristics, other genotypes can be included in the breeding population. Superior plants that are the products of these crosses are selfed and selected in successive generations. Each succeeding generation becomes more homogeneous as a result of self-pollination and selection. Typically, this method of breeding involves five or more generations of selfing and selection: $S_1 \rightarrow S_2$; $S_2 \rightarrow S_3$; $S_3 \rightarrow S_4$; $S_4 \rightarrow S_5$, etc. After at least five generations, the inbred plant is considered genetically pure.

Backcrossing can also be used to improve an inbred plant. Backcrossing transfers a specific desirable trait from one inbred or other source to an inbred that lacks that trait. This can be accomplished for example by first crossing a superior inbred (A) (recurrent parent) to a donor inbred (non-recurrent parent), which carries the appropriate gene(s) for the trait in question. The progeny of this cross are then mated back to the superior recurrent parent (A) followed by selection in the resultant progeny for the desired trait to be transferred from the non-recurrent parent. After five or more backcross generations with selection for the desired trait, the progeny are heterozygous for loci controlling the characteristic being transferred, but are like the superior parent for most or almost all other genes. The last backcross generation would be selfed to give pure breeding progeny for the gene(s) being transferred.

A single cross hybrid corn variety is the cross of two inbred plants, each of which has a genotype which complements the genotype of the other. The hybrid progeny of the first generation is designated F_1. Preferred F_1 hybrids are more vigorous than their inbred parents. This hybrid vigor, or heterosis, is manifested in many polygenic traits, including markedly improved higher yields, better stalks, better roots, better uniformity and better insect and disease resistance. In the development of hybrids only the F_1 hybrid plants are sought. An F_1 single cross hybrid is produced when two inbred plants are crossed. A double cross hybrid is produced from four inbred plants crossed in pairs (A×B and C×D) and then the two F_1 hybrids are crossed again (A×B)×(C×D).

The development of a hybrid corn variety involves three steps: (1) the selection of plants from various germplasm pools; (2) the selfing of the selected plants for several generations to produce a series of inbred plants, which, although different from each other, each breed true and are highly uniform; and (3) crossing the selected inbred plants with unrelated inbred plants to produce the hybrid progeny (F_1). During the inbreeding process in corn, the vigor of the plants decreases. Vigor is restored when two unrelated inbred plants are crossed to produce the hybrid progeny (F_1). An important consequence of the homozygosity and homogeneity of the inbred plants is that the hybrid between any two inbreds is always the same. Once the inbreds that give a superior hybrid have been identified, hybrid seed can be reproduced indefinitely as long as the homogeneity of the inbred parents is maintained. Conversely, much of the hybrid vigor exhibited by F_1 hybrids is lost in the next generation (F_2). Consequently, seed from hybrid varieties is not used for planting stock. It is not generally beneficial for farmers to save seed of F_1 hybrids. Rather, farmers purchase F_1 hybrid seed for planting every year.

North American farmers plant over 70 million acres of corn at the present time and there are extensive national and international commercial corn breeding programs. A continuing goal of these corn breeding programs is to develop high-yielding corn hybrids that are based on stable inbred plants that maximize the amount of grain produced and minimize susceptibility to environmental stresses. To accomplish this goal, the corn breeder must select and develop superior inbred parental plants for producing hybrids.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a corn plant designated 87DIA4. Also provided are corn plants having all the physiological and morphological characteristics of corn plant 87DIA4.

The inbred corn plant of the invention may further comprise, or have, a cytoplasmic factor that is capable of conferring male sterility. Parts of the corn plant of the present invention are also provided, such as, e.g., pollen obtained from an inbred plant and an ovule of the inbred plant.

The invention also concerns seed of the corn plant 87DIA4, which has been deposited with the ATCC. The invention thus provides inbred corn seed designated 87DIA4, and having ATCC Accession No. 203192.

The inbred corn seed of the invention may be provided as an essentially homogeneous population of inbred corn seed designated 87DIA4.

Essentially homogeneous populations of inbred seed are those that consist essentially of the particular inbred seed, and are generally purified free from substantial numbers of other seed, so that the inbred seed forms between about 90% and about 100% of the total seed, and preferably, between about 95% and about 100% of the total seed. Most preferably, an essentially homogeneous population of inbred corn seed will contain between about 98.5%, 99%, 99.5% and about 100% of inbred seed, as measured by seed grow outs.

In any event, even if a population of inbred corn seed was found, for some reason, to contain about 50%, or even about 20% or 15% of inbred seed, this would still be distinguished from the small fraction of inbred seed that may be found within a population of hybrid seed, e.g., within a bag of hybrid seed. In such a bag of hybrid seed offered for sale, the Governmental regulations require that the hybrid seed be at least about 95% of the total seed. In the practice of the present invention, the hybrid seed generally forms at least about 97% of the total seed. In the most preferred practice of the invention, the female inbred seed that may be found within a bag of hybrid seed will be about 1% of the total seed, or less, and the male inbred seed that may be found within a bag of hybrid seed will be negligible, i.e., will be on the order of about a maximum of 1 per 100,000, and usually less than this value.

The population of inbred corn seed of the invention is further particularly defined as being essentially free from hybrid seed. The inbred seed population may be separately grown to provide an essentially homogeneous population of inbred corn plant designated 87DIA4.

In another aspect, the present invention provides for single gene converted plants of 87DIA4. The single transferred gene may preferably be a dominant or recessive allele. Preferably, the single transferred gene will confer such traits as male sterility, herbicide resistance, insect resistance, resistance for bacterial, fungal, or viral disease, male fertility, enhanced nutritional quality, and industrial usage. The single gene may be a naturally occurring maize gene or a transgene introduced through genetic engineering techniques.

In another aspect, the present invention provides a tissue culture of regenerable cells of inbred corn plant 87DIA4. The tissue culture will preferably be capable of regenerating plants having the physiological and morphological characteristics of the foregoing inbred corn plant, and of regenerating plants having substantially the same genotype as the foregoing inbred corn plant. Preferably, the regenerable cells in such tissue cultures will be embryos, protoplasts, meristematic cells, callus, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks or stalks. Still further, the present invention provides corn plants regenerated from the tissue cultures of the invention.

In yet another aspect, the present invention provides processes for preparing corn seed or plants, which processes generally comprise crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is the inbred corn plant designated 87DIA4. These processes may be further exemplified as processes for preparing hybrid corn seed or plants, wherein a first inbred corn plant is crossed with a second, distinct inbred corn plant to provide a hybrid that has, as one of its parents, the inbred corn plant 87DIA4.

In a preferred embodiment, crossing comprises planting, in pollinating proximity, seeds of the first and second parent corn plant, and preferably, seeds of a first inbred corn plant and a second, distinct inbred corn plant; cultivating or growing the seeds of said first and second parent corn plants into plants that bear flowers; emasculating the male flowers of the first or second parent corn plant, i.e., treating the flowers so as to prevent pollen production, in order to produce an emasculated parent corn plant; allowing natural cross-pollination to occur between the first and second parent corn plants; and harvesting the seeds from the emasculated parent corn plant. Where desired, the harvested seed is grown to produce a corn plant or hybrid corn plant.

The present invention also provides corn seed and plants produced by a process that comprises crossing a first parent corn plant with a second parent corn plant, wherein at least one of the first or second parent corn plants is the inbred corn plant designated 87DIA4. In one embodiment, corn plants produced by the process are first generation (F_1) hybrid corn plants produced by crossing an inbred in accordance with the invention with another, distinct inbred. The present invention further contemplates seed of an F_1 hybrid corn plant.

In certain exemplary embodiments, the invention provides an F_1 hybrid corn plant and seed thereof, which hybrid corn plant is designated 4033843, having 871DIA4 as one inbred parent.

In yet a further aspect, the invention provides an inbred genetic complement of the corn plant designated 87DIA4. The phrase "genetic complement" is used to refer to the aggregate of nucleotide sequences, the expression of which sequences defines the phenotype of, in the present case, a corn plant, or a cell or tissue of that plant. An inbred genetic complement thus represents the genetic make up of an inbred cell, tissue or plant, and a hybrid genetic complement represents the genetic make up of a hybrid cell, tissue or plant. The invention thus provides corn plant cells that have a genetic complement in accordance with the inbred corn plant cells disclosed herein, and plants, seeds and diploid plants containing such cells.

Plant genetic complements may be assessed by genetic marker profiles, and by the expression of phenotypic traits that are characteristic of the expression of the genetic complement, e.g., isozyme typing profiles. Thus, such corn plant cells may be defined as having an RFLP genetic marker profile in accordance with the profile shown in Table 8, or a genetic isozyme typing profile in accordance with the profile shown in Table 9, or having both an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 8 and 9.

In another aspect, the present invention provides hybrid genetic complements, as represented by corn plant cells, tissues, plants and seeds, formed by the combination of a haploid genetic complement of an inbred corn plant of the invention with a haploid genetic complement of a second corn plant, preferably, another, distinct inbred corn plant. In another aspect, the present invention provides a corn plant regenerated from a tissue culture that comprises a hybrid genetic complement of this invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. DEFINITIONS

Barren Plants: Plants that are barren, i.e., lack an ear with grain, or have an ear with only a few scattered kernels.

Cg: *Colletotrichum graminicola* rating. Rating times 10 is approximately equal to percent total plant infection.

CLN: Corn Lethal Necrosis (combination of Maize (Chlorotic Mottle Virus and Maize Dwarf Mosaic virus) rating: numerical ratings are based on a severity scale where 1=most resistant to 9=susceptible.

Cn: *Corynebacterium nebraskense* rating. Rating times 10 is approximately equal to percent total plant infection.

Cz: *Cercospora zeae-maydis* rating. Rating times 10 is approximately equal to percent total plant infection.

Dgg: *Diatraea grandiosella* girdling rating (values are percent plants girdled and stalk lodged).

Dropped Ears: Ears that have fallen from the plant to the ground.

Dsp: Diabrotica species root ratings (1=least affected to 9=severe pruning).

Ear-Attitude: The attitude or position of the ear at harvest scored as upright, horizontal, or pendant.

Ear-Cob Color: The color of the cob, scored as white, pink, red, or brown.

Ear-Cob Diameter: The average diameter of the cob measured at the midpoint.

Ear-Cob Strength: A measure of mechanical strength of the cobs to breakage, scored as strong or weak.

Ear-Diameter: The average diameter of the ear at its midpoint.

Ear-Dry Husk Color: The color of the husks at harvest scored as buff, red, or purple.

Ear-Fresh Husk The color of the husks 1 to 2 weeks after pollination scored as Color: green, red, or purple.

Ear-Husk Bract: The length of an average husk leaf scored as short, medium, or long.

Ear-Husk Cover: The average distance from the tip of the ear to the tip of the husks. Minimum value no less than zero.

Ear-Husk Opening: An evaluation of husk tightness at harvest scored as tight, intermediate, or open.

Ear-Length: The average length of the ear.

Ear-Number Per The average number of ears per plant. Stalk:

Ear-Shank The average number of internodes on the ear shank. Internodes:

Ear-Shank Length: The average length of the ear shank.

Ear-Shelling Percent: The average of the shelled grain weight divided by the sum of the shelled grain weight and cob weight for a single ear.

Ear-Silk Color: The color of the silk observed 2 to 3 days after silk emergence scored as green-yellow, yellow, pink, red, or purple.

Ear-Taper (Shape): The taper or shape of the ear scored as conical, semi-conical, or cylindrical.

Ear-Weight: The average weight of an ear.

Early Stand: The percent of plants that emerge from the ground as determined in the early spring.

ER: Ear rot rating (values approximate percent ear rotted).

Final Stand Count: The number of plants just prior to harvest.

GDUs to Shed: The number of growing degree units (GDUs) or heat units required for an inbred line or hybrid to have approximately 50 percent of the plants shedding pollen as measured from time of planting. Growing degree units are calculated by the Barger Method, where the heat units for a 24-hour period are calculated as GDUs=[Maximum daily temperature+Minimum daily temperature)/2]-50. The highest maximum daily temperature used is 86 degrees Fahrenheit and the lowest minimum temperature used is 50 degrees Fahrenheit. GDUs to shed is then determined by summing the individual daily values from planting date to the date of 50 percent pollen shed.

GDUs to Silk: The number of growing degree units for an inbred line or hybrid to have approximately 50 percent of the plants with silk emergence as measured from time of planting. Growing degree units are calculated by the same methodology as indicated in the GDUs to shed definition.

Hc2: *Helminthosporium carbonum* race 2 rating. Rating times 10 is approximately equal to percent total plant infection.

Hc3: *Helminthosporium carbonum* race 3 rating. Rating times 10 is approximately equal to percent total plant infection.

Hm: *Helminthosporium maydis* race 0 rating. Rating times 10 is approximately equal to percent total plant infection.

Ht1: *Helminthosporium turcicum* race 1 rating. Rating times 10 is approximately equal to percent total plant infection.

Ht2: *Helminthosporium turcicum* race 2 rating. Rating times 10 is approximately equal to percent total plant infection.

HtG: +=Presence of Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection. --Absence of a Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection. +/-=Segregation of a Ht chlorotic-lesion type resistance. Rating times 10 is approximately equal to percent total plant infection.

Kernel-Aleurone The color of the aleurone scored as white, pink, tan, brown, Color: bronze, red, purple, pale purple, colorless, or variegated.

Kernel-Cap Color: The color of the kernel cap observed at dry stage, scored as white, lemon-yellow, yellow or orange.

Kernel-Endosperm The color of the endosperm scored as white, pale yellow, or Color: yellow.

Kernel-Endosperm The type of endosperm scored as normal, waxy, or opaque. Type:

Kernel-Grade: The percent of kernels that are classified as rounds.

Kernel-Length: The average distance from the cap of the kernel to the pedicel.

Kernel-Number Per The average number of kernels in a single row. Row:

Kernel-Pericarp The color of the pericarp scored as colorless, red-white crown, tan, Color: bronze, brown, light red, cherry red, or variegated.

Kernel-Row The direction of the kernel rows on the ear scored as straight, Direction: slightly curved, spiral, or indistinct (scattered).

Kernel-Row Number: The average number of rows of kernels on a single ear.

Kernel-Side Color: The color of the kernel side observed at the dry stage, scored as white, pale yellow, yellow, orange, red, or brown.

Kernel-Thickness: The distance across the narrow side of the kernel.

Kernel-Type: The type of kernel scored as dent, flint, or intermediate.

Kernel-Weight: The average weight of a predetermined number of kernels.

Kernel-Width: The distance across the flat side of the kernel.

Kz: *Kabatiella zeae* rating. Rating times 10 is approximately equal to percent total plant infection.

Leaf-Angle: Angle of the upper leaves to the stalk scored as upright (0 to 30 degrees), intermediate (30 to 60 degrees), or lax (60 to 90 degrees).

Leaf-Color: The color of the leaves 1 to 2 weeks after pollination scored as light green, medium green, dark green, or very dark green.

Leaf-Length: The average length of the primary ear leaf.

Leaf-Longitudinal A rating of the number of longitudinal creases on the leaf surface 1 Creases: to 2 weeks after pollination. Creases are scored as absent, few, or many.

Leaf-Marginal A rating of the waviness of the leaf margin 1 to 2 weeks after Waves: pollination. Rated as none, few, or many.

Leaf-Number: The average number of leaves of a mature plant. Counting begins with the cotyledonary leaf and ends with the flag leaf.

Leaf-Sheath A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks Anthocyanin: after pollination, scored as absent, basal-weak, basal-strong, weak or strong.

Leaf-Sheath A rating of the pubescence of the leaf sheath. Ratings are taken 1 Pubescence: to 2 weeks after pollination and scored as light, medium, or heavy.

Leaf-Width: The average width of the primary ear leaf measured at its widest point.

LSS: Late season standability (values times 10 approximate percent plants lodged in disease evaluation plots).

Moisture: The moisture of the grain at harvest.

On1: *Ostrinia nubilalis* 1st brood rating (1=resistant to 9=susceptible).

On2: *Ostrinia nubilalis* 2nd brood rating (1=resistant to 9=susceptible).

Relative Maturity: A maturity rating based on regression analysis. The regression analysis is developed by utilizing check hybrids and their previously established day rating versus actual harvest moistures. Harvest moisture on the hybrid in question is determined and that moisture value is inserted into the regression equation to yield a relative maturity.

Root Lodging: Root lodging is the percentage of plants that root lodge. A plant is counted as root lodged if a portion of the plant leans from the vertical axis by approximately 30 degrees or more.

Seedling Color: Color of leaves at the 6 to 8 leaf stage.

Seedling Height: Plant height at the 6 to 8 leaf stage.

Seedling Vigor: A visual rating of the amount of vegetative growth on a 1 to 9 scale, where 1 equals best. The score is taken when the average entry in a trial is at the fifth leaf stage.

Selection Index: The selection index gives a single measure of hybrid's worth based on information from multiple traits. One of the traits that is almost always included is yield. Traits may be weighted according to the level of importance assigned to them.

Sr: *Sphacelotheca reiliana* rating is actual percent infection.

Stalk-Anthocyanin: A rating of the amount of anthocyanin pigmentation in the stalk. The stalk is rated 1 to 2 weeks after pollination as absent, basal-weak, basal-strong, weak, or strong.

Stalk-Brace Root The color of the brace roots observed 1 to 2 weeks after pollination Color: as green, red, or purple.

Stalk-Diameter: The average diameter of the lowest visible internode of the stalk.

Stalk-Ear Height: The average height of the ear measured from the ground to the point of attachment of the ear shank of the top developed ear to the stalk.

Stalk-Internode The direction of the stalk internode observed after pollination as Direction: straight or zigzag.

Stalk-Internode The average length of the internode above the primary ear. Length:

Stalk Lodging: The percentage of plants that did stalk lodge. Plants are counted as stalk lodged if the plant is broken over or off below the ear.

Stalk-Nodes With The average number of nodes having brace roots per plant. Brace Roots:

Stalk-Plant Height: The average height of the plant as measured from the soil to the tip of the tassel.

Stalk-Tillers: The percent of plants that have tillers. A tiller is defined as a secondary shoot that has developed as a tassel capable of shedding pollen.

Staygreen: Staygreen is a measure of general plant health near the time of black layer formation (physiological maturity). It is usually recorded at the time the ear husks of most entries within a trial have turned a mature color. Scoring is on a 1 to 9 basis where 1 equals best.

STR: Stalk rot rating (values represent severity rating of 1=25 percent of inoculated internode rotted to 9=entire stalk rotted and collapsed).

SVC: Southeastern Virus Complex combination of Maize Chlorotic Dwarf Virus and Maize Dwarf Mosaic Virus) rating; numerical ratings are based on a severity scale where 1=most resistant to 9=susceptible (1988 reactions are largely Maize Dwarf Mosaic Virus reactions).

Tassel-Anther Color: The color of the anthers at 50 percent pollen shed scored as green-yellow, yellow, pink, red, or purple.

Tassel-Attitude: The attitude of the tassel after pollination scored as open or compact.

Tassel-Branch Angle: The angle of an average tassel branch to the main stem of the tassel scored as upright (less than 30 degrees), intermediate (30 to 45 degrees), or lax (greater than 45 degrees).

Tassel-Branch The average number of primary tassel branches. Number:

Tassel-Glume Band: The closed anthocyanin band at the base of the glume scored as present or absent.

Tassel-Glume Color: The color of the glumes at 50 percent shed scored as green, red, or purple.

Tassel-Length: The length of the tassel measured from the base of the bottom tassel branch to the tassel tip.

Tassel-Peduncle The average length of the tassel peduncle, measured from the base Length: of the flag leaf to the base of the bottom tassel branch.

Tassel-Pollen Shed: A visual rating of pollen shed determined by tapping the tassel and observing the pollen flow of approximately five plants per entry. Rated on a 1 to 9 scale where 9=sterile, 1=most pollen.

Tassel-Spike Length: The length of the spike measured from the base of the top tassel branch to the tassel tip.

Test Weight: The measure of the weight of the grain in pounds for a given volume (bushel) adjusted to 15.5 percent moisture.

Yield: Yield of grain at harvest adjusted to 15.5 percents moisture.

II. OTHER DEFINITIONS

Allele is any of one or more alternative forms of a gene, all of which alleles relate to one trait or characteristic. In a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes.

Backcrossing is a process in which a breeder repeatedly crosses hybrid progeny back to one of the parents, for example, a first generation hybrid (F_1) with one of the parental genotypes of the F1 hybrid.

Chromatography is a technique wherein a mixture of dissolved substances are bound to a solid support followed by passing a column of fluid across the solid support and varying the composition of the fluid. The components of the mixture are separated by selective elution.

Crossing refers to the mating of two parent plants.

Cross-pollination refers to fertilization by the union of two gametes from different plants.

Diploid refers to a cell or organism having two sets of chromosomes.

Electrophoresis is a process by which particles suspended in a fluid are moved under the action of an electrical field, and thereby separated according to their charge and molecular weight. This method of separation is well known to those skilled in the art and is typically applied to separating various forms of enzymes and of DNA fragments produced by restriction endonucleases.

Emasculate refers to the removal of plant male sex organs.

Enzymes are organic catalysts that can exist in various forms called isozymes.

F_1 Hybrid refers to the first generation progeny of the cross of two plants.

Genetic Complement refers to an aggregate of nucleotide sequences, the expression of which sequences defines the phenotype in corn plants, or components of plants including cells or tissue.

Genotype refers to the genetic constitution of a cell or organism.

Haploid refers to a cell or organism having one set of the two sets of chromosomes in a diploid.

Isozymes are one of a number of enzymes which catalyze the same reaction(s) but differ from each other, e.g., in primary structure and/or electrophoretic mobility. The differences between isozymes are under single gene, codominant control. Consequently, electrophoretic separation to produce band patterns can be equated to different alleles at the, DNA level. Structural differences that do not alter charge cannot be detected by this method.

Isozyme typing profile refers to a profile of band patterns of isozymes separated by electrophoresis that can be equated to different alleles at the DNA level.

Linkage refers to a phenomenon wherein alleles on the same chromosome tend to segregate together more often than expected by chance if their transmission was independent.

Marker is a readily detectable phenotype, preferably inherited in codominant fashion (both alleles at a locus in a diploid heterozygote are readily detectable), with no environmental variance component, i.e., heritability of 1.

87DIA4 refers to the corn plant from which seeds having ATCC Accession No. 203192 were obtained, as well as plants grown from those seeds.

Phenotype refers to the detectable characteristics of a cell or organism, which characteristics are the manifestation of gene expression.

Quantitative Trait Loci (QTL) refer to genetic loci that control to some degree numerically representable traits that are usually continuously distributed.

Regeneration refers to the development of a plant from tissue culture.

RFLP genetic marker profile refers to a profile of band patterns of DNA fragment lengths typically separated by agarose gel electrophoresis, after restriction endonuclease digestion of DNA.

Self-pollination refers to the transfer of pollen from the anther to the stigma of the same plant.

Single Gene Converted (Conversion) Plant refers to plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single gene transferred into the inbred via the backcrossing technique.

Tissue Culture refers to a composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

III. INBRED CORN PLANT 87DIA4

In accordance with one aspect of the present invention, there is provided a novel inbred corn plant, designated 87DIA4. Inbred corn plant 87DIA4 is a yellow, dent corn inbred that can be compared to inbred corn plants 2FACC, 3AZA1, and AQA3, all of which are proprietary inbreds of DEKALB Genetics Corporation. 87DIA4 differs significantly (at the 1%, 5%, or 10% level) from these inbred lines in several aspects (Table 1, Table 2, and Table 3).

TABLE 1

COMPARISON OF 87DIA4 WITH 2FACC

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
2FACC	0.4	0.6	29.5	62.0	23.9	67.1	1.3	1482.6	1481.5	5.8	77.4
DIFF	-0.1	-0.5	-5.2	0.4	-6.1	-10.1	-1.1	-119.5	-124.4	2.4	-12.3
# LOC	15	13	8	15	14	8	14	8	8	13	13
P VALUE	0.88	0.65	0.00**	0.84	0.00**	0.00**	0.40	0.00**	0.00**	0.36	0.01*

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

TABLE 2

COMPARISON OF 87DIA4 WITH 3AZA1

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
3AZA1	1.1	1.6	23.6	62.0	15.9	58.4	0.1	1322.6	1321.2	8.4	41.1
DIFF	-0.8	-1.5	0.7	0.4	1.9	-1.4	0.1	40.5	35.9	-0.2	24.0
# LOC	15	13	8	15	14	8	14	8	8	13	13
P VALUE	0.41	0.19	0.66	0.84	0.13	0.48	0.94	0.00**	0.03*	0.94	0.00**

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

TABLE 3

COMPARISON OF 87DIA4 WITH AQA3

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
87DIA4	0.3	0.1	24.3	62.4	17.8	57.0	0.2	1363.1	1357.1	8.2	65.1
AQA3	0.4	0.5	25.9	62.7	14.4	58.1	1.0	1356.2	1348.6	15.2	35.7
DIFF	-0.1	-0.4	-1.6	-0.3	3.3	-1.1	-0.8	6.9	8.5	-7.0	29.4

TABLE 3-continued

COMPARISON OF 87DIA4 WITH AQA3

INBRED	BARREN %	DROP %	EHT INCH	FINAL	MST %	PHT INCH	RTL %	SHED GDU	SILK GDU	STL %	YLD BU
# LOC	15	13	8	15	14	8	14	8	8	10	13
P VALUE	0.86	0.72	0.34	0.86	0.00**	0.58	0.56	0.64	0.61	0.01*	0.00**

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
Legend Abbreviations:
BARREN % = Barren Plants (percent)
DROP % = Dropped Ears (percent)
EHT INCH = Ear Height (inches)
FINAL = Final Stand
MST % = Moisture (percent)
PHT INCH = Plant Height (inches)
RTL % = Root Lodging (percent)
SHED GDU = GDUs to Shed
SILK GDU = GDUS to Silk
STL % = Stalk Lodging (percent)
YLD BU = Yield (bushels/acre)

A. ORIGIN AND BREEDING HISTORY

Inbred plant 87DIA4 was derived from the cross between a line derived from 2FACC and 3AZA1.

87DIA4 shows uniformity and stability within the limits of environmental influence for the traits described hereinafter in Table 4. 87DIA4 has been self-pollinated and ear-rowed a sufficient number of generations with careful attention paid to uniformity of plant type to ensure homozygosity and phenotypic stability. No variant traits have been observed or are expected in 87DIA4.

A deposit of 2500 seeds of plant designated 87DIA4 has been made with the American Type Culture Collection (ATCC), Rockville Pike, Bethesda, Md. on Sep. 11, 1998. Those deposited seeds have been assigned Accession No. 203192. The deposit was made in accordance with the terms and provisions of the Budapest Treaty relating to deposit of microorganisms and is made for a term of at least thirty (30) years and at least five (05) years after the most recent request for the furnishing of a sample of the deposit was received by the depository, or for the effective term of the patent, whichever is longer, and will be replaced if it becomes non-viable during that period.

Inbred corn plants can be reproduced by planting such inbred seeds, growing the resulting corn plants under self-pollinating or sib-pollinating conditions with adequate isolation using standard techniques well known to an artisan skilled in the agricultural arts. Seeds can be harvested from such a plant using standard, well known procedures.

The origin and breeding history of inbred plant 87DIA4 can be summarized as follows:

Summer 1988 The cross 2FACC and AQA3 was made. Both inbreds are proprietary to DEKALB Genetics Corporation.

Winter 1988 S0 seed was grown (nursery row 67-51).

Summer 1989 S1 seed was grown (nursery rows 4-25 to 4-38).

Winter 1989 S2 seed was grown ear-to-row (nursery row 649-62).

Summer 1990 S3 seed was grown ear-to-row (nursery row 130-15).

Winter 1990 S4 seed was grown ear-to-row (nursery row C23-23).

Summer 1991 S5 seed was grown ear-to-row (nursery row 222-67).

Summer 1992 S6 seed was grown ear-to-row (nursery row 418-56).

Summer 1993 S7 seed was grown ear-to-row (nursery rows 346-32 to 346-39). Seed from all rows was bulked to form 87DIA4.

B. PHENOTYPIC DESCRIPTION

In accordance with another aspect of the present invention, there is provided a corn plant having the physiological and morphological characteristics of corn plant 87DIA4. A description of the physiological and morphological characteristics of corn plant 87DIA114 is presented in Table 4.

TABLE 4

MORPHOLOGICAL TRAITS FOR THE 87DIA4 PHENOTYPE

CHARACTERISTIC	87DIA4	2FACC	3AZA1	AQA3
1. STALK				
Diameter (width) cm	1.9	2.2	2.0	2.2
Anthocyanin	Absent	Absent	Absent	Absent
Nodes with Brace Roots	1.4	1.9	2.0	1.5
Brace Root Color	Red	Purple	—	Green
Internode Direction	Straight	Straight	Straight	Straight

TABLE 4-continued

MORPHOLOGICAL TRAITS FOR THE 87DIA4 PHENOTYPE

CHARACTERISTIC	87DIA4	2FACC	3AZA1	AQA3
Internode Length cm.	10.2	12.7	14.0	11.3
2. LEAF				
Color	Med Green	Med Green	Med Green	Med Green
Length cm.	68.0	71.1	66.2	67.9
Width cm.	10.0	8.7	7.9	8.3
Sheath Anthocyanin	Weak	Weak	Weak	Absent
Sheath Pubescence	Medium	Light	Medium	Medium
Marginal Waves	Few	Few	Few	Few
Longitudinal Creases	Absent	Absent	—	Few
3. TASSEL				
Attitude	Compact	Compact	—	Open
Length cm.	29.5	26.7	33.0	33.0
Spike Length cm.	19.5	19.1	24.4	23.1
Peduncle Length cm.	2.9	5.2	3.6	3.6
Branch Number	4.5	7.7	3.8	5.5
Anther Color	Red	Pink	Tan	Grn-Yellow
Glume Color	Green	Green	Green	Green
Glume Band	Absent	Absent	Absent	Absent
4. EAR				
Silk Color	Pink	Tan	Grn-Yellow	Grn-Yellow
Number Per Stalk	1.1	1.1	1.6	1.4
Position (attitude)	Upright	Upright	Pendant	Upright
Length cm.	15.6	13.9	16.4	16.3
Shape	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Diameter cm.	3.8	4.2	3.4	3.6
Weight gm.	99.1	116.3	89.8	93.1
Shank Length cm.	16.5	14.8	20.7	14.9
Husk Bract	Short	Short	Short	Short
Husk Cover cm.	3.4	6.6	1.9	3.2
Husk Opening	Tight	Intermediate	—	Intermediate
Husk Color Fresh	Green	Lt Green	Green	Lt Green
Husk Color Dry	Buff	Buff	Buff	Buff
Cob Diameter cm.	2.3	2.6	1.7	2.1
Cob Color	Red	Red	Red	Red
Shelling Percent	85.1	81.4	85.8	85.0
5. KERNEL				
Row Number	14.0	14.7	12.3	15.1
Number Per row	31.4	25.5	32.2	33.2
Row Direction	Curved	Curved	Curved	Curved
Type	Dent	Dent	Dent	Dent
Cap Color	Yellow	Yellow	Yellow	Lemon Yellow
Side Color	Yellow	Deep Yellow	Orange	Orange
Length (depth) mm.	10.2	10.7	9.2	9.6
Width mm.	8.1	8.1	7.3	7.1
Thickness	4.3	4.3	4.2	3.8
Weight of 1000K gm.	281.5	280.7	223.8	173.7
Endosperm Type	Normal	Normal	Normal	Normal
Endosperm Color	Yellow	Yellow	Yellow	Yellow

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

IV. ADDITIONAL INBRED CORN PLANTS

The inbred corn plant 171KI3 has been employed with the corn plant of the present invention in order to produce an exemplary hybrid. A description of the physiological and morphological characteristics of this corn plant is presented herein at Table 5. Additional information for this inbred corn plant is presented in co-pending U.S. patent application Ser. No. 08/795,403, filed Feb. 5, 1997, the disclosure of which application is specifically incorporated herein by reference.

TABLE 5

MORPHOLOGICAL TRAITS FOR THE 171KI3 PHENOTYPE

CHARACTERISTIC	171KI3	01CSI2	01IBH2	3IIH6
1. STALK				
Diameter (width) cm.	2.2	2.4	2.1	2.3

TABLE 5-continued

MORPHOLOGICAL TRAITS FOR THE 171KI3 PHENOTYPE

CHARACTERISTIC	171KI3	01CSI2	01IBH2	3IIH6
Anthocyanin	Absent	Absent	Absent	Absent
Nodes With Brace Roots	0.9	1.8	1.1	0.7
Brace Root Color	Green	Green	Green	—
Internode Direction	Straight	Straight	Straight	Straight
Internode Length cm.	15.9	12.8	14.4	13.1
2. LEAF				
Color	Med Green	—	Med Green	Med Green
Width cm.	9.7	8.9	8.9	8.0
Marginal Waves	Few	Few	Few	Few
3. TASSEL				
Length cm.	42.6	31.2	33.6	35.3
Spike Length cm.	22.9	23.2	23.1	25.2
Peduncle Length cm.	9.6	3.9	8.2	7.6
Branch Number	9.1	7.4	7.8	12.9
Anther Color	Purple	Grn-Yellow	Grn-Yellow	—
Glume Color	Purple	Green	Green	—
Glume Band	Present	Absent	Absent	Absent
4. EAR	Pink	Pink	—	Red
Silk Color	1.0	1.0	1.0	1.4
Number Per Stalk	Upright	—	—	Upright
Position (attitude)	14.6	16.0	14.6	15.6
Length cm.	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Shape	4.0	3.8	4.0	3.9
Diameter cm.	104.9	100.6	103.2	107.6
Weight gm.	10.3	14.1	10.1	9.6
Shank Length cm.	Short	Short	Short	Short
Husk Bract	6.4	2.5	4.4	3.7
Husk Cover cm.	Green	Green	Green	Green
Husk Color Fresh	Buff	Buff	Buff	Buff
Husk Color Dry	2.3	2.4	2.3	2.1
Cob Diameter cm.	Red	—	Red	Red
Cob Color	87.7	80.6	89.0	83.3
Shelling Percent				
5. KERNEL	14.6	14.8	16.3	15.3
Row Number	32.1	27.1	29.3	29.7
Number Per Row	Curved	Curved	Curved	Curved
Row Direction	Dent	—	Dent	—
Type	Yellow	Yellow	Yellow	Yellow
Cap Color	Deep Yellow	—	Orange	
Side Color	11.1	9.4	10.9	10.3
Length (depth) mm.	7.8	8.0	7.4	7.8
Width mm.	3.9	5.2	4.4	4.2
Thickness	269.0	252.4	233.0	247.8
Weight of 1000K gm.	Normal	Normal	Normal	Normal
Endosperm Type	Yellow	Yellow	Yellow	Yellow
Endosperm Color				

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

V. SINGLE GENE CONVERSIONS

When the term inbred corn plant is used in the context of the present invention, this also includes any single gene conversions of that inbred. The term single gene converted plant as used herein refers to those corn plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single gene transferred into the inbred via the backcrossing technique. Backcrossing methods can be used with the present invention to improve or introduce a characteristic into the inbred. The term backcrossing as used herein refers to the repeated crossing of a hybrid progeny back to one of the parental corn plants for that inbred. The parental corn plant which contributes the gene for the desired characteristic is termed the nonrecurrent or donor parent. This terminology refers to the fact that the nonrecurrent parent is used one time in the backcross protocol and therefore does not recur. The parental corn plant to which the gene or genes from the nonrecurrent parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol (Poehlman & Sleper, 1994; Fehr, 1987). In a typical backcross protocol, the original inbred of interest (recurrent parent) is crossed to a second inbred (nonrecurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a corn plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the

converted plant, in addition to the single transferred gene from the nonrecurrent parent.

The selection of a suitable recurrent parent is an important step for a successful backcrossing procedure. The goal of a backcross protocol is to alter or substitute a single trait or characteristic in the original inbred. To accomplish this, a single gene of the recurrent inbred is modified or substituted with the desired gene from the nonrecurrent parent, while retaining essentially all of the rest of the desired genetic, and therefore the desired physiological and morphological, constitution of the original inbred. The choice of the particular nonrecurrent parent will depend on the purpose of the backcross, one of the major purposes is to add some commercially desirable, agronomically important trait to the plant. The exact backcrossing protocol will depend on the characteristic or trait being altered to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele may also be transferred. In this instance it may be necessary to introduce a test of the progeny to determine if the desired characteristic has been successfully transferred.

Many single gene traits have been identified that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques. Single gene traits may or may not be transgenic, examples of these traits include but are not limited to, male sterility, waxy starch, herbicide resistance, resistance for bacterial, fungal, or viral disease, insect resistance, male fertility, enhanced nutritional quality, industrial usage, yield stability and yield enhancement. These genes are generally inherited through the nucleus. Some known exceptions to this are the genes for male sterility, some of which are inherited cytoplasmically, but still act as single gene traits. Several of these single gene traits are described in U.S. Ser. No. 07/113,561, filed Aug. 25, 1993, the disclosure of which is specifically hereby incorporated by reference.

Direct selection may be applied where the single gene acts as a dominant trait. An example might be the herbicide resistance trait. For this selection process, the progeny of the initial cross are sprayed with the herbicide prior to the backcrossing. The spraying eliminates any plants which do not have the desired herbicide resistance characteristic, and only those plants which have the herbicide resistance gene are used in the subsequent backcross. This process is then repeated for all additional backcross generations.

The waxy characteristic is an example of a recessive trait. In this example, the progeny resulting from the first backcross generation (BC1) must be grown and selfed. A test is then run on the selfed seed from the BC1 plant to determine which BC1 plants carried the recessive gene for the waxy trait. In other recessive traits, additional progeny testing, for example growing additional generations such as the BC1S1 may be required to determine which plants carry the recessive gene.

VI. ORIGIN AND BREEDING HISTORY OF AN EXEMPLARY SINGLE GENE CONVERTED PLANT

85DGD1 MLms is a single gene conversion of 85DGD1 to cytoplasmic male sterility. 85DGD1 MLms was derived using backcross methods. 85DGD1 (a proprietary inbred of DEKALB Genetics Corporation) was used as the recurrent parent and MLms, a germplasm source carrying ML cytoplasmic sterility, was used as the nonrecurrent parent. The breeding history of the single gene converted inbred 85DGD1 MLms can be summarized as follows:

Hawaii Nurseries Planting Date Apr. 2, 1992 Made up S-O: Female row 585 male row 500

Hawaii Nurseries Planting Date Jul. 15, 1992 S-O was grown and plants were backcrossed times 85DGD1 (rows 444' 443)

Hawaii Nurseries Planting Date Bulked seed of the BC1 was grown and Nov. 18, 1992 backcrossed times 85DGD1 (rows V3-27' V3-26)

Hawaii Nurseries Planting Date Apr. 2, 1993 Bulked seed of the BC2 was gown and backcrossed times 85DGD1 (rows 37' 36)

Hawaii Nurseries Planting Date Jul. 14, 1993 Bulked seed of the BC3 was grown and backcrossed times 85DGD1 (rows 99' 98)

Hawaii Nurseries Planting Date Bulked seed of BC4 was grown and backcrossed Oct. 28, 1993 times 85DGD1 (rows KS-63' KS-62)

Summer 1994 A single ear of the BC5 was grown and backcrossed times 85DGD1 (MC94-822' MC94-822-7)

Winter 1994 Bulked seed of the BC6 was grown and backcrossed times 85DGD1 (3Q-1' 3Q-2)

Summer 1995 Seed of the BC7 was bulked and named 85DGD1 MLms.

VII. TISSUE CULTURE AND IN VITRO REGENERATION OF CORN PLANTS

A further aspect of the invention relates to tissue culture of corn plants designated 87DIA4. As used herein, the term "tissue culture" indicates a composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant. Exemplary types of tissue cultures are protoplasts, calli, plant clumps, and plant cells that are intact in plants or parts of plants, such as embryos, pollen, flowers, kernels, ears, cobs, leaves, husks, stalks, roots, root tips, anthers, silk and the like. In a preferred embodiment, tissue culture is embryos, protoplast, meristematic cells, pollen, leaves or anthers. Means for preparing and maintaining plant tissue culture are well known in the art. By way of example, a tissue culture comprising organs such as tassels or anthers, has been used to produce regenerated plants. (See, U.S. patent applications Ser. No. 07/992,637, filed Dec. 18, 1992 and 07/995,938, filed Dec. 21, 1992, now issued as U.S. Pat. No. 5,322,789, issued Jun. 21, 1994, the disclosures of which are incorporated herein by reference).

VIII. TASSEL/ANTHER CULTURE

Tassels contain anthers which in turn enclose microspores. Microspores develop into pollen. For anther/microspore culture, if tassels are the plant composition., they are preferably selected at a stage when the microspores are uninucleate, that is, include only one, rather than 2 or 3 nuclei. Methods to determine the correct stage are well known to those killed in the art and include mitramycin fluorescent staining (Pace et al., 1987), trypan blue (preferred) and acetocarmine squashing. The mid-uninucleate microspore stage has been found to be the developmental stage most responsive to the subsequent methods disclosed to ultimately produce plants.

Although microspore-containing plant organs such as tassels can generally be pretreated at any cold temperature below about 25° C., a range of 4 to 25° C. is preferred, and a range of 8 to 14° C. is particularly preferred. Although other temperatures yield embryoids and regenerated plants, cold temperatures produce optimum response rates compared to pretreatment at temperatures outside the preferred range. Response rate is measured as either the number of embryoids or the number of regenerated plants per number of microspores initiated in culture.

Although not required, when tassels are employed as the plant organ, it is generally preferred to sterilize their surface.

Following surface sterilization of the tassels, for example, with a solution of calcium hypochloride, the anthers are removed from about 70 to 150 spikelets (small portions of the tassels) and placed in a preculture or pretreatment medium. Larger or smaller amounts can be used depending on the number of anthers.

When one elects to employ tassels directly, tassels are preferably pretreated at a cold temperature for a predefined time, preferably at 10° C. for about 4 days. After pretreatment of a whole tassel at a cold temperature, dissected anthers are further pretreated in an environment that diverts microspores from their developmental pathway. The function of the preculture medium is to switch the developmental program from one of pollen development that of embryoid/callus development. An embodiment of such an environment in the form of a preculture medium includes a sugar alcohol, for example mannitol or sorbitol, inositol or the like. An exemplary synergistic combination is the use of mannitol at a temperature of about 10° C. for a period ranging from about 10 to 14 days. In a preferred embodiment, 3 ml of 0.3 M mannitol combined with 50 mg/l of ascorbic acid, silver nitrate and colchicine is used for incubation of anthers at 10° C. for between 10 and 14 days. Another embodiment is to substitute sorbitol for mannitol. The colchicine produces chromosome doubling at this early stage. The chromosome doubling agent is preferably only present at the preculture stage.

It is believed that the mannitol or other similar carbon structure or environmental stress induces starvation and functions to force microspores to focus their energies on entering developmental stages. The cells are unable to use, for example, mannitol as a carbon source at this stage. It is believed that these treatments confuse the cells causing them to develop as embryoids and plants from microspores. Dramatic increases in development from these haploid cells, as high as 25 embryoids in 10^4 microspores, have resulted from using these methods.

In embodiments where microspores are obtained from anthers, microspores can be released from the anthers into an isolation medium following the mannitol preculture step. One method of release is by disruption of the anthers, for example, by chopping the anthers into pieces with a sharp instrument, such as a razor blade, scalpel or Waring blender. The resulting mixture of released microspores, anther fragments and isolation medium are then passed through a filter to separate microspores from anther wall fragments. An embodiment of a filter is a mesh, more specifically, a nylon mesh of about 112 mm pore size. The filtrate which results from filtering the microspore-containing solution is preferably relatively free of anther fragments, cell walls and other debris.

In a preferred embodiment, isolation of microspores is accomplished at a temperature below about 25° C. and, preferably at a temperature of less than about 15° C. Preferably, the isolation media, dispersing tool (e.g., razor blade) funnels, centrifuge tubes and dispersing container (e.g., petri dish) are all maintained at the reduced temperature during isolation. The use of a precooled dispersing tool to isolate maize microspores has been reported (Gaillard et al., 1991).

Where appropriate and desired, the anther filtrate is then washed several times in isolation medium. The purpose of the washing and centrifugation is to eliminate any toxic compounds which are contained in the non-microspore part of the filtrate and are created by the chopping process. The centrifugation is usually done at decreasing spin speeds, for example, 1000, 750, and finally 500 rpms.

The result of the foregoing steps is the preparation of a relatively pure tissue culture suspension of microspores that are relatively free of debris and anther remnants.

To isolate microspores, an isolation media is preferred. An isolation media is used to separate microspores from the anther walls while maintaining their viability and embryogenic potential. An illustrative embodiment of an isolation media includes a 6 percent sucrose or maltose solution combined with an antioxidant such as 50 mg/l of ascorbic acid, 0.1 mg/l biotin and 400 mg/l of proline, combined with 10 mg/l of nicotinic acid and 0.5 mg/l $AgNO_3$. In another embodiment, the biotin and proline are omitted.

An isolation media preferably has a higher antioxidant level where used to isolate microspores from a donor plant (a plant from which a plant composition containing a microspore is obtained) that is field grown in contrast to greenhouse grown. A preferred level of ascorbic acid in an isolation medium is from about 50 mg/l to about 125 mg/l and, more preferably from about 50 mg/l to about 100 mg/l.

One can find particular benefit in employing a support for the microspores during culturing and subculturing. Any support that maintains the cells near the surface can be used. The microspore suspension is layered onto a support, for example by pipetting. There are several types of supports which are suitable and are within the scope of the invention. An illustrative embodiment of a solid support is a TRANSWELL® culture dish. Another embodiment of a solid support for development of the microspores is a bilayer plate wherein liquid media is on top of a solid base. Other embodiments include a mesh or a millipore filter. Preferably, a solid support is a nylon mesh in the shape of a raft. A raft is defined as an approximately circular support material which is capable of floating slightly above the bottom of a tissue culture vessel, for example, a petri dish, of about a 60 or 100 mm size, although any other laboratory tissue culture vessel will suffice. In an illustrative embodiment, a raft is about 55 mm in diameter.

Culturing isolated microspores on a solid support, for example, on a 10 mm pore nylon raft floating on 2.2 ml of medium in a 60 mm petri dish, prevents microspores from sinking into the liquid medium and thus avoiding low oxygen tension. These types of cell supports enable the serial transfer of the nylon raft with its associated microspore/embryoids ultimately to full strength medium containing activated charcoal and solidified with, for example, GELRITE™ (solidifying agent). The charcoal is believed to absorb toxic wastes and intermediaries. The solid medium allows embryoids to mature.

The liquid medium passes through the mesh while the microspores are retained and supported at the medium-air interface. The surface tension of the liquid medium in the petri dish causes the raft to float. The liquid is able to pass through the mesh: consequently, the microspores stay on top. The mesh remains on top of the total volume of liquid medium. An advantage of the raft is to permit diffusion of nutrients to the microspores. Use of a raft also permits transfer of the microspores from dish to dish during subsequent subculture with minimal loss, disruption or disturbance of the induced embryoids that are developing. The rafts represent an advantage over the multi-welled TRANSWELL® plates, which are commercially available from COSTAR, in that the commercial plates are expensive. Another disadvantage of these plates is that to achieve the serial transfer of microspores to subsequent media, the membrane support with cells must be peeled off the insert in the wells. This procedure does not produce as good a yield nor as efficient transfers, as when a mesh is used as a vehicle for cell transfer.

The culture vessels can be further defined as either (1) a bilayer 60 mm petri plate wherein the bottom 2 ml of medium are solidified with 0.7 percent agarose, overlaid with 1 mm of liquid containing the microspores; (2) a nylon mesh raft wherein a wafer of nylon is floated on 1.2 ml of medium and 1 ml of isolated microspores is pipetted on top; or (3) TRANSWELL® plates wherein isolated microspores are pipetted onto membrane inserts which support the microspores at the surface of 2 ml of medium.

After the microspores have been isolated, they are cultured in a low strength anther culture medium until about the 50 cell stage when they are subcultured onto an embryoid/callus maturation medium. Medium is defined at this stage as any combination of nutrients that permit the microspores to develop into embryoids or callus. Many examples of suitable embryoid/callus promoting media are well known to those skilled in the art. These media will typically comprise mineral salts, a carbon source, vitamins, growth regulations. A solidifying agent is optional. A preferred embodiment of such a media is referred to by the inventor as the "D medium" which typically includes 6N1 salts, $AgNO_3$ and sucrose or maltose.

In an illustrative embodiment, 1 ml of isolated microspores are pipetted onto a 10 mm nylon raft and the raft is floated on 1.2 ml of medium "D", containing sucrose or, preferably maltose. Both calli and embryoids can develop. Calli are undifferentiated aggregates of cells. Type I is a relatively compact, organized and slow growing callus. Type II is a soft, friable and fast-growing one. Embryoids are aggregates exhibiting some embryo-like structures. The embryoids are preferred for subsequent steps to regenerating plants. Culture medium "D" is an embodiment of medium that follows the isolation medium and replaces it. Medium "D" promotes growth to an embryoid/callus. This medium comprises 6N1 salts at ⅛ the strength of a basic stock solution, (major components) and minor components, plus 12 percent sucrose or, preferably 12 percent maltose, 0.1 mg/l B1, 0.5 mg/l nicotinic acid, 400 mg/l proline and 0.5 mg/l silver nitrate. Silver nitrate is believed to act as an inhibitor to the action of ethylene. Multi-cellular structures of approximately 50 cells each generally arise during a period of 12 days to 3 weeks. Serial transfer after a two week incubation period is preferred.

After the petri dish has been incubated for an appropriate period of time, preferably two weeks, in the dark at a predefined temperature, a raft bearing the dividing microspores is transferred serially to solid based media which promotes embryo maturation. In an illustrative embodiment, the incubation temperature is 30° C. and the mesh raft supporting the embryoids is transferred to a 100 mm petri dish containing the 6N1-TGR-4P medium, an "anther culture medium." This medium contains 6N1 salts, supplemented with 0.1 mg/l TIBA, 12 percent sugar (sucrose, maltose or a combination thereof), 0.5 percent activated charcoal, 400 mg/l proline, 0.5 mg/l B, 0.5 mg/l nicotinic acid, and 0.2 percent GELRITE™ (solidifying agent) and is capable of promoting the maturation of the embryoids. Higher quality embryoids, that is, embryoids which exhibit more organized development, such as better shoot meristem formation without precocious germination were typically obtained with the transfer to full strength medium compared to those resulting from continuous culture using only, for example, the isolated microspore culture (IMC) Medium "D." The maturation process permits the pollen embryoids to develop further in route toward the eventual regeneration of plants. Serial transfer occurs to full strength solidified 6N1 medium using either the nylon raft, the TRANSWELL® membrane or bilayer plates, each one requiring the movement of developing embryoids to permit further development into physiologically more mature structures.

In an especially preferred embodiment, microspores are isolated in an isolation media comprising about 6 percent maltose, cultured for about two weeks in an embryoid/calli induction medium comprising about 12 percent maltose and then transferred to a solid medium comprising about 12 percent sucrose.

At the point of transfer of the raft after about two weeks incubation, embryoids exist on a nylon support. The purpose of transferring the raft with the embryoids to a solidified medium after the incubation is to facilitate embryo maturation. Mature embryoids at this point are selected by visual inspection indicated by zygotic embryo-like dimensions and structures and are transferred to the shoot initiation medium. It is preferred that shoots develop before roots, or that shoots and roots develop concurrently. If roots develop before shoots, plant regeneration can be impaired. To produce solidified media, the bottom of a petri dish of approximately 100 mm is covered with about 30 ml of 0.2 percent GELRITE™ (solidifying agent) solidified medium. A sequence of regeneration media are used for whole plant formation from the embryoids.

During the regeneration process, individual embryoids are induced to form plantlets. The number of different media in the sequence can vary depending on the specific protocol used. Finally, a rooting medium is used as a prelude to transplanting to soil. When plantlets reach a height of about 5 cm, they are then transferred to pots for further growth into flowering plants in a greenhouse by methods well known to those skilled in the art.

Plants have been produced from isolated microspore cultures by methods disclosed herein, including self-pollinated plants. The rate of embryoid induction was much higher with the synergistic preculture treatment consisting of a combination of stress factors, including a carbon source which can be capable of inducing starvation, a cold temperature and colchicine, than has previously been reported. An illustrative embodiment of the synergistic combination of treatments leading to the dramatically improved response rate compared to prior methods, is a temperature of about 10° C., mannitol as a carbon source, and 0.05 percent colchicine.

The inclusion of ascorbic acid, an anti-oxidant, in the isolation medium is preferred for maintaining good microspore viability. However, there seems to be no advantage to including mineral salts in the isolation medium. The osmotic potential of the isolation medium was maintained optimally with about 6 percent sucrose, although a range of 2 percent to 12 percent is within the scope of this invention.

In an embodiment of the embryoid/callus organizing media, mineral salts concentration in IMC Culture Media "D" is (⅛x), the concentration which is used also in anther culture medium. The 6N1 salts major components have been modified to remove ammonium nitrogen. Osmotic potential in the culture medium is maintained with about 12 percent sucrose and about 400 mg/l proline. Silver nitrate (0.5 mg/l) was included in the medium to modify ethylene activity. The preculture media is further characterized by having a pH of about 5.7 to 6.0. Silver nitrate and vitamins do not appear to be crucial to this medium but do improve the efficiency of the response.

Whole anther cultures can also be used in the production of monocotyledonous plants from a plant culture system. There are some basic similarities of anther culture methods

and microspore culture methods with regard to the media used. A difference from isolated microspore cultures is that undisrupted anthers are cultured, so that a support, eg., a nylon mesh support, is not needed. The first step in developing the anther cultures is to incubate tassels at a cold temperature. A cold temperature is defined as less than about 25° C. More specifically, the incubation of the tassels is preferably performed at about 10° C. A range of 8 to 14° C. is also within the scope of the invention. The anthers are then dissected from the tassels, preferably after surface sterilization using forceps, and placed on solidified medium. An example of such a medium is designated by the inventors as 6N1-TGR-P4.

The anthers are then treated with environmental conditions that area combinations of stresses that are capable of diverting microspores from gametogenesis to embryogenesis. It is believed that the stress effect of sugar alcohols in the preculture medium, for example, mannitol, is produced by inducing starvation at the predefined temperature. In one embodiment, the incubation pretreatment is for about 14 days at 10° C. It was found that treating the anthers in addition with a carbon structure, an illustrative embodiment being a sugar alcohol, preferably, mannitol, produces dramatically higher anther culture response rates as measured by the number of eventually regenerated plants, than by treatment with either cold treatment or mannitol alone. These results are particularly surprising in light of teachings that cold is better than mannitol for these purposes, and that warmer temperatures interact with mannitol better.

To incubate the anthers, they are floated on a preculture medium which diverts the microspores from gametogenesis, preferably on a mannitol carbon structure,, more specifically, 0.3 M of mannitol plus 50 mg/l of ascorbic acid. 3 ml is about the total amount in a dish, for example, a tissue culture dish, more specifically, a 60 mm petri dish. Anthers are isolated from about 120 spikelets for one dish yields about 360 anthers.

Chromosome doubling agents can be used in the preculture media for anther cultures. Several techniques for doubling chromosome number (Jensen, 1974; Wan et al., 1989) have been described. Colchicine is one of the doubling agents. However, developmental abnormalities arising from in vitro cloning are further enhanced by colchicine treatments, and previous reports indicated that colchicine is toxic to microspores. The addition of colchicine in increasing concentrations during mannitol pretreatment prior to anther culture and microspore culture has achieved improved percentages.

An illustrative embodiment of the combination of a chromosome doubling agent and preculture medium is one which contains colchicine. In a specific embodiment, the colchicine level is preferably about 0.05 percent. The anthers remain in the mannitol preculture medium with the additives for about 10 days at 10° C. Anthers are then placed on maturation media, for example, that designated 6N1-TGR-P4, for 3 to 6 weeks to induce embryoids. If the plants are to be regenerated from the embryoids, shoot regeneration medium is employed, as in the isolated microspore procedure described in the previous sections. Other regeneration media can be used sequentially to complete regeneration of whole plants.

The anthers are then exposed to embryoid/callus promoting medium, for example, that designated 6N1-TGR-P4 to obtain callus or embryoids. The embryoids are recognized by identification visually of embryonic-like structures. At this stage, the embryoids are transferred serially to a series of regeneration media. In an illustrative embodiment, :he shoot initiation medium comprises BAP (6-benzyl-aminopurine) and NAA (naphthalene acetic acid). Regeneration protocols for isolated microspore cultures and anther cultures are similar.

IX. OTHER CULTURES AND REGENERATION

The present invention contemplates a corn plant regenerated from a tissue culture of an inbred (e.g., 87DIA4) or hybrid plant (e.g., 4033843) of the present invention. As is well known in the art, tissue culture of corn can be used for the in vitro regeneration of a corn plant. By way of example, a process of tissue culturing and regeneration of corn is described in European Patent Application, publication 160, 390, the disclosure of which is incorporated by reference. Corn tissue culture procedures are also described in Green & Rhodes (1982) and Duncan et al., (1985). The study by Duncan et al. (1985) indicates that 97 percent of cultured plants produced calli capable of regenerating plants. Subsequent studies have shown that both inbreds and hybrids produced 91 percent regenerable calli that produced plants.

Other studies indicate that non-traditional tissues are capable of producing somatic embryogenesis and plant regeneration. See, e.g., Songstad et al. (1988); Rao et al. (1986); and Conger et al. (1987), the disclosures of which are incorporated herein by reference. Regenerable cultures may be initiated from immature embryos as described in PCT publication WO 95/06128, the disclosure of which is incorporated herein by reference.

Briefly, by way of example, to regenerate a plant of this invention, cells are selected following growth in culture. Where employed, cultured cells are preferably grown either on solid supports or in the form of liquid suspensions as set forth above. In either instance, nutrients are provided to the cells in the form of media, and environmental conditions are controlled. There are many types of tissue culture media comprising amino acids, salts, sugars, hormones and vitamins. Most of the media employed to regenerate inbred and hybrid plants have some similar components, the media differ in the composition and proportions of their ingredients depending on the particular application envisioned. For example, various cell types usually grow in more than one type of media, but exhibit different growth rates and different morphologies, depending on the growth media. In some media, cells survive but do not divide. Various types of media suitable for culture of plant cells have been previously described and discussed above.

An exemplary embodiment for culturing recipient corn cells in suspension cultures includes using embryogenic cells in Type II (Armstrong & Green, 1985; (Gordon-Kamm et al., 1990) callus, selecting for small (10 to 30 m) isodiametric, cytoplasmically dense cells, growing the cells in suspension cultures with hormone containing media, subculturing into a progression of media to facilitate development of shoots and roots, and finally, hardening the plant and readying it metabolically for growth in soil.

Meristematic cells (i.e., plant cells capable of continual cell division and characterized by an undifferentiated cytological appearance, normally found at growing points or tissues in plants such as root tips, stem apices, lateral buds, etc.) can be cultured.

Embryogenic calli are produced essentially as described in PCT Publication WO 95/06128. Specifically, inbred plants or plants from hybrids produced from crossing an inbred of the present invention with another inbred are grown to flowering in a greenhouse. Explants from at least one of the following F_1 tissues: the immature tassel tissue, intercalary meristems and leaf bases, apical meristems, immature ears and immature embryos are placed in an

initiation medium which contain MS salts, supplemented with thiamine, agar, and sucrose. Cultures are incubated in the dark at about 23° C. All culture manipulations and selections are performed with the aid of a dissecting microscope.

After about 5 to 7 days, cellular outgrowths are observed from the surface of the explants. After about 7 to 21 days, the outgrowths are subcultured by placing them into fresh medium of the same composition. Some of the intact immature embryo explants are placed on fresh medium. Several subcultures later (after about 2 to 3 months) enough material is present from explants for subdivision of these embryogenic calli into two or more pieces.

Callus pieces from different explants are not mixed. After further growth and subculture (about 6 months after embryogenic callus initiation), there are usually between 1 and 100 pieces derived ultimately from each selected explant. During this time of culture expansion, a characteristic embryogenic culture morphology develops as a result of careful selection at each subculture. Any organized structures resembling roots or root primordia are discarded. Material known from experience to lack the capacity for sustained growth is also discarded (translucent, watery, embryogenic structures). Structures with a firm consistency resembling at least in part the scutelum of the in vivo embryo are selected.

The callus is maintained on agar-solidified MS or N6-type media. A preferred hormone is 2,4-D. A second preferred hormone is dicamba. Visual selection of embryo-like structures is done to obtain subcultures. Transfer of material other than that displaying embryogenic morphology results in loss of the ability to recover whole plants from the callus.

Cell suspensions are prepared from the calli by selecting cell populations that appear homogeneous macroscopically. A portion of the friable, rapidly growing embryogenic calli is inoculated into MS or N6 Medium containing 2,4-D or dicamba. The calli in medium are incubated at about 27° C. on a gyrotary shaker in the dark or in the presence of low light. The resultant suspension culture is transferred about once every three to seven days, preferably every three to four days, by taking about 5 to 10 ml of the culture and introducing this inoculum into fresh medium of the composition listed above.

For regeneration, embryos which appear on the callus surface are selected and regenerated into whole plants by transferring the embryogenic structure, into a sequence of solidified media which include decreasing concentrations of 2,4-D or other auxins. Other hormones which can be used in culture media include dicamba, NAA, ABA, BAP, and 2-NCA. The reduction is relative to the concentration used in culture maintenance media. Plantlets are regenerated from these embryos by transfer to a hormone-free medium, subsequently transferred to soil, and grown to maturity.

Progeny are produced by taking pollen and selfing, backcrossing or sibling regenerated plants by methods well known to those skilled in the arts. Seeds are collected from the regenerated plants.

X. PROCESSES OF PREPARING CORN PLANTS AND THE CORN PLANTS PRODUCED BY SUCH CROSSES

The present invention also provides a process of preparing a novel corn plant and a corn plant produced by such a process. In accordance with such a process, a first parent corn plant is crossed with a second parent corn plant wherein at least one of the first and second corn plants is the inbred corn plant 87DIA4. In one embodiment, a corn plant prepared by such a process is a first generation F_1 hybrid corn plant prepared by a process wherein both the first and second parent corn plants are inbred corn plants.

Corn plants (*Zea mays L.*) can be crossed by either natural or mechanical techniques. Natural pollination occurs in corn when wind blows pollen from the tassels to the silks that protrude from the tops of the incipient ears. Mechanical pollination can be effected either by controlling the types of pollen that can blow onto the silks or by pollinating by hand.

In a preferred embodiment, crossing comprises the steps of:

(a) planting in pollinating proximity seeds of a first and a second parent corn plant, and preferably, seeds of a first inbred corn plant and a second, distinct inbred corn plant;

(b) cultivating or growing the seeds of the first and second parent corn plants into plants that bear flowers;

(c) emasculating flowers of either the first or second parent corn plant, i.e., treating the flowers so as to prevent pollen production, in order to produce an emasculated parent corn plant;

(d) allowing natural cross-pollination to occur between the first and second parent corn plant;

(e) harvesting seeds produced on the emasculated parent corn plant; and, where desired,

(f) growing the harvested seed into a corn plant, or preferably, a hybrid corn plant.

Parental plants are planted in pollinating proximity to each other by planting the parental plants in alternating rows, in blocks or in any other convenient planting pattern. Plants of both parental parents are cultivated and allowed to grow until the time of flowering. Advantageously, during this growth stage, plants are in general treated with fertilizer and, or other agricultural chemicals as considered appropriate by the grower.

At the time of flowering, in the event that plant 87DIA4, is employed as the male parent, the tassels of the other parental plant are removed from all plants employed as the female parental plant. The detasseling can be achieved manually but also can be done by machine, if desired.

The plants are then allowed to continue to grow and natural cross-pollination occurs as a result of the action of wind, which is normal in the pollination of grasses, including corn. As a result of the emasculation of the female parent plant, all the pollen from the male parent plant 87DIA4 is available for pollination because tassels, and thereby pollen bearing flowering parts, have been previously removed from all plants of the inbred plant being used as the female in the hybridization. Of course, during this hybridization procedure, the parental varieties are grown such that they are isolated from other corn fields to minimize or prevent my accidental contamination of pollen from foreign sources. These isolation techniques are well within the skill of those skilled in this art.

Both parental inbred plants of corn may be allowed to continue to grow until maturity or the male rows may be destroyed after flowering is complete. Only the ears from the female inbred parental plants are harvested to obtain seeds of a novel F_1 hybrid. The novel F_1 hybrid seed produced can then be planted in a subsequent growing season with the desirable characteristics in terms of F_1 hybrid corn plants providing improved grain yields and the other desirable characteristics disclosed herein, being achieved.

Alternatively, in another embodiment, both first and second parent corn plants can come from the same inbred corn plant, i.e., from the inbred designated 87DIA4. Thus, any corn plant produced using a process of the present invention and inbred corn plant 87DIA4, is contemplated by this invention. As used herein, crossing can mean selfing,

backcrossing, crossing to another or the same inbred, crossing to populations, and the like. All corn plants produced using the present inbred corn plant 87DIA4 as a parent are within the scope of this invention.

The utility of the inbred plant 87DIA4 also extends to crosses with other species. Commonly, suitable species will be of the family Graminaceae, and especially of the genera Zea, Tripsacum, Coix, Schlerachne, Polytoca, Chionachne, and Trilobachne, of the tribe Maydeae. Of these, Zea and Tripsacum, are most preferred. Potentially suitable for crosses with 87DIA4 can be the various varieties of grain sorghum, *Sorghum bicolor* (L.) Moench.

A. F_1 HYBRID CORN PLANT AND SEED PRODUCTION

Where the inbred corn plant 87DIA4 is crossed with another, different, corn inbred, a first generation (F_1) corn hybrid plant is produced. Both a F_1 hybrid corn plant and a seed of that F_1 hybrid corn plant are contemplated as aspects of the present invention.

Inbred 87DIA4 has been used to prepare an F_1 hybrid corn plant, designated 4033843.

The goal of a process of producing an F_1 hybrid is to manipulate the genetic complement of corn to generate new combinations of genes which interact to yield new, or improved traits (phenotypic characteristics). A process of producing an F_1 hybrid typically begins with the production of one or more inbred plants. Those plants are produced by repeated crossing of ancestrally related corn plants to try and concentrate certain genes within the inbred plants. The production of inbred 87DIA4 has been set forth hereinbefore.

Corn has a diploid phase which means two conditions of a gene (two alleles) occupy each locus (position on a chromosome). If the alleles are the same at a locus, there is said to be homozygosity. If they are different, there is said to be heterozygosity. In a completely inbred plant, all loci are homozygous. Because many loci when homozygous are deleterious to the plant, in particular leading to reduced vigor, less kernels, weak and/or poor growth, production of inbred plants is an unpredictable and arduous process. Under some conditions, heterozygous advantage at some loci effectively bars perpetuation of homozygosity.

Inbreeding requires coddling and sophisticated manipulation by human breeders. Even in the extremely unlikely event inbreeding rather than crossbreeding occurred in natural corn, achievement of complete inbreeding cannot be expected in nature due to well known deleterious effects of homozygosity and the large number of generations the plant would have to breed in isolation. The reason for the breeder to create inbred plants is to have a known reservoir of genes whose gametic transmission is at least somewhat predictable.

The development of inbred plants generally requires at least about 5 to 7 generations of selfing. Inbred plants are then cross-bred in an attempt to develop improved F_1 hybrids. Hybrids are then screened and evaluated in small scale field trials. Typically, about 10 to 15 phenotypic traits, selected for their potential commercial value, are measured. A selection index of the most commercially important traits is used to help evaluate hybrids. FACT, an acronym for Field Analysis Comparison Trial (strip trials), is an on-farm testing program employed by DEKALB Plant Genetics to perform the final evaluation of the commercial potential of a product.

During the next several years, a progressive elimination of hybrids occurs based on more detailed evaluation of their phenotype. Eventually, strip trials (FACT) are conducted to formally compare the experimental hybrids being developed with other hybrids, some of which were previously developed and generally are commercially successful. That is, comparisons of experimental hybrids are made to competitive hybrids to determine if there was any advantage to further commercial development of the experimental hybrids. Examples of such comparisons are presented in Section B, hereinbelow.

When the inbred parental plant 87DIA4 is crossed with another inbred plant to yield a hybrid (such as the hybrid 4033843), the original inbred can serve as either the maternal or paternal plant. For many crosses, the outcome is the same regardless of the assigned sex of the parental plants.

However, there is often one of the parental plants that is preferred as the maternal plant because of increased seed yield and production characteristics. Some plants produce tighter ear husks leading to more loss, for example due to rot. There can be delays in silk formation which deleteriously affect timing of the reproductive cycle for a pair of parental inbreds. Seed coat characteristics can be preferable in one plant. Pollen can be shed better by one plant. Other variables can also affect preferred sexual assignment of a particular cross.

B. F_1 HYBRID COMPARISONS

As mentioned in Section A, hybrids are progressively eliminated following detailed evaluations of their phenotype, including formal comparisons with other commercially successful hybrids. Strip trials are used to compare the phenotypes of hybrids grown in as many environments as possible. They are performed in many environments to assess overall performance of the new hybrids and to select optimum growing conditions. Because the corn is grown in close proximity, environmental factors that affect gene expression, such as moisture, temperature, sunlight and pests, are minimized. For a decision to be made that a hybrid is worth making commercially available, it is not necessary that the hybrid be better than all other hybrids. Rather, significant improvements must be shown in at least some, traits that would create improvements in some niches.

Examples of such comparative data are set forth hereinbelow in Table 6, which presents a comparison of performance data for the hybrid 4033843, a hybrid made with 87DIA4 as one parent, versus a selected hybrid of commercial value (DK442).

All the data in Table 6 represents results across years and locations for research and/or strip trials. The "NTEST" represents the number of paired observations in designated tests at locations around the United States.

TABLE 6

COMPARATIVE DATA FOR 4033843

HYBRID	NTEST	SI %C	YLD BU	MST PTS	STL %	RTL %	DRP %	FLSTD %M	SV RAT

TABLE 6-continued

COMPARATIVE DATA FOR 4033843										
4033843	R 93	110.3	156.3	19.9	5.2	1.4	0.1	101.0	4.1
DK442		99.0	147.7	19.8	7.1	5.2	0.1	100.9	4.3
DEV		11.3**	8.6**	0.1	-1.9**	-3.8**	0.0	0.2	-0.1

HYBRID	NTEST	ELSTD % M	PHT INCH	EHT INCH	BAR %	SG RAT	TST LBS	FGDU	ESTR DAYS
4033843	R 93	104.4	89.8	40.9		5.0	54.2	1214.0	94.0
DK442		102.9	90.2	43.9		3.1	53.4	1251.0	93.9
DEV		1.5+	-0.4	-3.0**		1.8**	0.8**	-36.9**	0.1

Significance levels are indicated as:
+ = 10 percent,
* = 5 percent,
** = 1 percent.
LEGEND ABBREVIATIONS:
HYBD = Hybrid
TEST = Research/FACT
SI % C = Selection Index (percent of check)
YLD BU = Yield (bushels/acre)
MST PTS = Moisture
STL % = Stalk Lodging (percent)
RTL % = Root Lodging (percent)
DRP % = Dropped Ears (percent)
FLSTD % M = Final Stand (percent of test mean)
SV RAT = Seedling Vigor Rating
ELSTD % M = Early Stand (percent of test mean)
PHT INCH = Plant Height (inches)
EHT INCH = Ear Height (inches)
BAR % = Barren Plants (percent)
SG RAT = Staygreen Rating
TST LBS = Test Weight (pounds)
FGDU = GDUs to Shed
ESTR DAYS = Estimated Relative Maturity (days)

As can be seen in Table 6, the hybrid 4033843 has significantly higher yield with comparable moisture content when compared to a successful commercial hybrid. Significant differences are also shown in Table 6 for many other traits.

C. PHYSICAL DESCRIPTION OF F_1 HYBRIDS

The present invention also provides F_1 hybrid corn plants derived from the corn plant 87DIA4. Physical characteristics of exemplary hybrids are set forth in Table 7, which concerns 4033843, which has 87DIA4 as one inbred parent. An explanation of terms used in Table 7 can be found in the Definitions, set forth herein above.

TABLE 7

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996

	CHARACTERISTIC	VALUE
1.	STALK	
	Diameter (width) cm	2.6
	Anthocyanin	Absent
	Nodes with Brace Roots	1.5
	Brace Root Color	Red
	Internode Direction	Straight
	Internode Length cm.	16.0
2.	LEAF	
	Color	Med Green
	Length cm.	79.9
	Width cm.	10.7
	Sheath Anthocyanin	Absent
	Sheath Pubescence	Medium
	Marginal Waves	Medium
	Longitudinal Creases	Few

TABLE 7-continued

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996

	CHARACTERISTIC	VALUE
3.	TASSEL	
	Attitude	Compact
	Length cm.	48.1
	Spike Length cm.	27.4
	Peduncle Length cm.	12.1
	Branch Number	7.5
	Anther Color	Red
	Glume Color	Purple
	Glume Band	Absent
4.	EAR	
	Silk Color	Tan
	Number Per Stalk	1.1
	Position (attitude)	Upright
	Length cm.	20.7
	Shape	Semi-conical
	Diameter cm.	4.7
	Weight gm.	222.8
	Shank Length cm.	18.7
	Husk Bract	Short
	Husk Opening	Open
	Husk Color Fresh	Green
	Husk Color Dry	Buff
	Cob Diameter cm.	2.4
	Cob Color	Red
	Shelling Percent	88.6
5.	KERNEL	
	Row Number	15.4
	Number Per row	42.6
	Row Direction	Straight
	Type	Dent

TABLE 7-continued

MORPHOLOGICAL TRAITS FOR THE 4033843 PHENOTYPE YEAR OF DATA: 1996	
CHARACTERISTIC	VALUE
Cap Color	Yellow
Side Color	Deep Yellow
Length (depth) mm.	12.0
Width mm.	7.9
Thickness	4.1
Weight of 1000K gm.	307.0
Endosperm Type	Normal
Endosperm Color	Yellow

*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention. Substantially equivalent refers to quantitative traits that when compared do not show statistical differences of their means.

XI. GENETIC COMPLEMENTS

In another aspect, the present invention provides a genetic complement of a plant of this invention. In one embodiment, therefore, the present invention contemplates an inbred genetic complement of the inbred corn plant designated 87DIA4. In another embodiment, the present invention contemplates a hybrid genetic complement formed by the combination of a haploid genetic complement from 87DIA4 and another haploid genetic complement. Means for determining a genetic complement are well-known in the art.

As used herein, the phrase "genetic complement" means an aggregate of nucleotide sequences, the expression of which sequences defines the phenotype of a corn plant or a cell or tissue of that plant. By way of example, a corn plant is genotyped to determine the array of the inherited markers it possesses. Markers are alleles at a single locus. They are preferably inherited in codominant fashion so that the presence of both alleles at a diploid locus is readily detectable, and they are free of environmental variation, i.e., their heritability is 1. This genotyping is preferably performed on at least one generation of the descendant plant for which the numerical value of the quantitative trait or traits of interest are also determined. The array of single locus genotypes is expressed as a profile of marker alleles, two at each locus. The marker allelic composition of each locus can be either homozygous or heterozygous. Homozygosity is a condition where both alleles at a locus are characterized by the same nucleotide sequence. Heterozygosity refers to different conditions of the gene at a locus. Markers that are used for purposes of this invention include restriction fragment length polymorphisms (RFLPs) and isozymes.

A plant genetic complement can be defined by genetic marker profiles that can be considered "fingerprints" of a genetic complement. For purposes of this invention, markers are preferably distributed evenly throughout the genome to increase the likelihood they will be near a quantitative trait loci (QTL) of interest (e.g., in tomatoes, Helentjaris et al., U.S. Pat. No. 5,385,835, Nienhuis et al., 1987). These profiles are partial projections of a sample of genes. One of the uses of markers in general is to exclude, or alternatively include, potential parents as contributing to offspring.

Phenotypic traits characteristic of the expression of a genetic complement of this invention are distinguishable by electrophoretic separation of DNA sequences cleaved by various restriction endonucleases. Those traits (genetic markers) are termed RFLPs (restriction fragment length polymorphisms).

Restriction fragment length polymorphisms (RFLPs) are genetic differences detectable by DNA fragment lengths, typically revealed by agarose gel electrophoresis, after restriction endonuclease digestion of DNA. There are large numbers of restriction endonucleases available, characterized by their nucleotide cleavage sites and their source, e.g., Eco RI. Variations in RFLPs result from nucleotide base pair differences which alter the cleavage sites of the restriction endonucleases, yielding different sized fragments.

Means for performing RFLP analyses are well known in the art. Restriction fragment length polymorphism analyses reported herein were conducted by Linkage Genetics. This service is available to the public on a contractual basis. Probes were prepared to the fragment sequences, these probes being complementary to the sequences thereby being capable of hybridizing to them under appropriate conditions well known to those ,,killed in the art. These probes were labeled with radioactive isotopes or fluorescent dyes for ease of detection. After the fragments were separated by size, they were identified by the probes. Hybridization with a unique cloned sequence permits the identification of a specific chromosomal region (locus). Because all alleles at a locus are detectable, RFLPs are codominant alleles, thereby satisfying a criteria for a genetic marker. They differ from some other types of markers, e.g., from isozymes, in that they reflect the primary DNA sequence, they are not products of transcription or translation. Furthermore, different RFLP genetic marker profiles result from different arrays of restriction endonucleases.

The RFLP genetic marker profile of each of the parental inbreds and exemplary resultant hybrids were determined. Because an inbred is essentially homozygous at all relevant loci, an inbred should, in almost all cases, have only one allele at each locus. In contrast, a diploid genetic marker profile of a hybrid should be the sum of those parents, e.g., if one inbred parent had the allele A at a particular locus, and the other inbred parent had B, the hybrid is AB by inference. Subsequent generations of progeny produced by selection and breeding are anticipated to be of genotype A, B, or AB for that locus position. When the F1 plant is used to produce an inbred, the locus should be either A or B for that position. Surprisingly, it has been observed that in certain instances, novel RFLP genotypes arise during the breeding process. For example, a genotype of C is observed at a particular locus position from the cross of parental inbreds with A and B at that locus. Such a novel RFLP genotype is observed for the 87DIA4, at least, for the RFLP markers M5213S and M8B2369S, as shown in Table 8. These novel RFLP markers further define the 87DIA4 inbred from the parental inbreds from which it was derived. An RFLP genetic marker profile of 87DIA4 is presented in Table 8.

TABLE 8

RFLP PROFILE OF 87DIA4				
PROBE/ENZYME	87DIA4	2FACC	3AZA1	AQA3
M0264H	D	G	G	D
M0306H	A	A	—	A
M0445E	C	B	B	C
M1120S	F	—	D	F
M1234H	D	D	E	E
M1236H	A	A	—	A
M1238H	A	A	F	K
M1401E	C	C	C	A
M1406H	A	—	B	B
M1447H	B	B	E	B
M1B725E	B	B	C	C
M2239H	A	A	C	C
M2297H	A	A	E	A

TABLE 8-continued

RFLP PROFILE OF 87DIA4

PROBE/ENZYME	87DIA4	2FACC	3AZA1	AQA3
M2298E	C	B	C	C
M2402H	E	E	E	E
M3212S	A	A	B	A
M3247E	D	B	D	D
M3257S	B	B	B	B
M3296H	D	A	D	D
M3432H	A	I	A	A
M3446S	C	B	C	C
M3457E	E	E	E	E
M4386H	B	B	A	A
M4396H	H	H	F	F
M4444H	B	B	A	A
M4UMC19H	A	A	A	A
M4UMC31S	D	A	B	D
M5213S	B	A	B	A
M5295E	C	D	C	C
M5408H	A	A	A	A
M5579S	B	B	B	B
M5UMC95H	A	A	B	B
M6223E	C	C	C	C
M6252H	D	—	D	E
M6280H	E	E	A	A
M6373E	A	E	A	A
M7263E	A	C	A	A
M7391H	C	C	A	A
M7392S	C	C	B	C
M7455H	A	A	C	C
M8110S	C	C	C	C
M8114E	B	B	E	E
M8268H	B	B	B	B
M8585H	A	A	A	A
M8B2369S	B	D	B	D
M8UMC48E	C	C	C	C
M9209E	C	C	A	A
M9266S	A	A	C	C
M9B713S	A	A	B	B
M2UMC34H	D	D	D	—
M6UMC85H	A	A	A	—
M9UMC94H	E	E	B	—
M3UM121X	C	C	C	—
M0UMC130	C	H	—	—

*Probes used to detect RFLPs are from Linkage Genetics, 1515 West 2200 South, Suite C, Salt Lake City, Utah 84119.

Another aspect of this invention is a plant genetic complement characterized by a genetic isozyme typing profile. Isozymes are forms of proteins that are distinguishable, for example, on starch gel electrophoresis, usually by charge and/or molecular weight. The techniques and nomenclature for isozyme analysis are described in, Stuber et al. (1988), which is incorporated by reference.

A standard set of loci can be used as a reference set. Comparative analysis of these loci is used to compare the purity of hybrid seeds, to assess the increased variability in hybrids compared to inbreds, and to determine the identity of seeds, plants, and plant parts. In this respect, an isozyme reference set can be used to develop genotypic "fingerprints."

Table 9 lists the identifying numbers of the alleles at isozyme loci types, and represents the exemplary genetic isozyme typing profile for 87DIA4.

TABLE 9

ISOZYME PROFILE OF 87DIA4

	ISOZYME ALLELE			
LOCUS	87DIA4	2FACC	3AZA1	AQA3
Acph1	2	2	4	4
Adh1	4	4	4	4
Cat3	9	9	9	9
Got1	4	4	4	4
Got2	4	4	4	4
Got3	4	4	4	4
Idh1	4	4	4	4
Idh2	6	6	6	6
Mdh1	6	6	6*	6
Mdh2	3.5	3.5	6	3
Mdh3	16	16	16	16
Mdh4	12	12	12	12
Mdh5	12	12	12	12
Pgm1	9	9	9	9
Pgm2	4	4	4	4
6Pgd1	3.8	3.8	3.8	3.8
6Pgd2	5	5	5	5
Phi1	4	4	4	5

*Allele is probably a 6, but null cannot be ruled out.

The present invention also contemplates a hybrid genetic complement formed by the combination of a haploid genetic complement of the corn plant 87DIA4 with a haploid genetic complement of a second corn plant. Means for combining a haploid genetic complement from the foregoing inbred with another haploid genetic complement can be any method hereinbefore for producing a hybrid plant from 87DIA4. It is also contemplated that a hybrid genetic complement can be prepared using in vitro regeneration of a tissue culture of a hybrid plant of this invention.

A hybrid genetic complement contained in the seed of a hybrid derived from 87DIA4 is a further aspect of this invention. Exemplary hybrid genetic complements are the genetic complements of the hybrid 4033843.

Table 10 shows the identifying numbers of the alleles for the hybrid 4033843, which are exemplary RFLP genetic marker profiles for hybrids derived from the inbred of the present invention. Table 10 concerns 4033843, which has 87DIA4 as one inbred parent.

TABLE 10

RFLP PROFILE FOR 4033843

Probe/Enzyme Combination	Allelic Pair
M0264H	DH
M0306H	AA
M0445E	BC
M1120S	EF
M1234H	AD
M1238H	AE
M1401E	AC
M1406H	AB
M1447H	BB
M1B725E	BB
M2239H	AD
M2297H	AC
M2298E	CC
M2402H	EE
M3212S	AC
M3257S	AB
M3296H	CD
M3432H	AA
M3446S	CF
M3457E	EE
M4386H	BD

TABLE 10-continued

RFLP PROFILE FOR 4033843	
Probe/Enzyme Combination	Allelic Pair
M4396E	HH
M4444H	AB
M4UMC39H	AA
M4UMC31S	AD
M5213S	AB
M5408H	AA
M6223E	BC
M6252H	AD
M6280H	EG
M6373E	AE
M7263E	AA
M7391H	AC
M7392S	AC
M7455H	AB
M8110S	AC
M8114E	BB
M8268H	BL
M8585H	AB
M8B2369S	BB
M8UMC48E	CC
M9209E	AC
M9266S	AA
M9B713S	AA
M2UMC34H	DF
M9UMC94H	EE
M3UM121X	CD
M0UMC130	CC

*Probes used to detect RFLPs are from Linkage Genetics, 1515 West 2200 South, Suite C, Salt Lake City, Utah 84119.

The exemplary hybrid genetic complements of hybrid 4033843 may also be assessed by genetic isozyme typing profiles using a standard set of loci as a reference, set, using, e.g., the same, or a different, set of loci to those described above. Table 11 lists the identifying numbers of the alleles at isozyme loci types and presents the exemplary genetic isozyme typing profile for the hybrid 4033843, which is an exemplary hybrid derived from the inbred of the present invention. Table 11 concerns 4033843, which has 87DIA4 as one inbred parent.

TABLE 11

ISOZYME GENOTYPE FOR HYBRID 4033843	
LOCUS	ISOZYME ALLELES
Acph1	2
Adh1	4
Cat3	9
Got1	4
Got2	4
Got3	4
Idh1	4
Idh2	6
Mdh1	6
Mdh2	3.5
Mdh3	16
Mdh4	12
Mdh5	12
Pgm1	9
Pgm2	4
6-Pgd1	3.8
6-Pgd2	5
Phi1	4

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of the foregoing illustrative embodiments, it will be apparent to those of skill in the art that variations, changes, modifications and alterations may be applied to the composition, methods, and in the steps or in the sequence of steps of the methods described herein, without departing, from the true concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

Armstrong & Green, "Establishment and Maintenance of Friable Embryogenic Maize Callus and the Involvement of L-Proline," *Planta,* 164:207–214, 1985.

Conger et al., "Somatic Embryogenesis from Cultured Leaf Segments of *Zea Mays,*" *Plant Cell Reports,* 6:345–347, 1987.

Duncan et al., "The Production of Callus Capable of Plant Regeneration from Immature Embryos of Numerous *Zea Mays* Genotypes," *Planta,* 165:322–332, 1985.

Fehr (ed.), *Principles of Cultivar Development,* Vol. 1: Theory and Technique, pp. 360–376, 1987.

Gaillard et al., "Optimization of Maize Microspore Isolation and Culture Condition for Reliable Plant Regeneration," *Plant Cell Reports,* 10(2):55, 1991.

Gordon-Kamm et al., "Transformation of Maize Cells and Regeneration of Fertile Transgenic Plants," *The Plant Cell,* 6:603–618, 1990.

Green & Rhodes, "Plant Regeneration in Tissue Cultures of Maize," *Maize for Biological Research,* Plant Molecular Biology Association, pp. 367–372, 1992.

Jensen, "Chromosome Doubling Techniques in Haploids," Haploids and Higher Plants—*Advances and Potentials, Proceedings of the First International Symposium,* University of Guelph, Jun. 10–14, 1974.

Nienhuis et al., "Restriction Fragment Length Polymorphism Analysis of Loci Associated with Insect Resistance in Tomato," *Crop Science,* 27:797–803, 1987.

Pace et al., "Anther Culture of Maize and the Visualization of Embryogenic Microspores by Fluorescent Microscopy," *Theoretical and Applied Genetics,* 73:83 63–869, 1987.

Poehlman & Sleper (eds), *Breeding Field Crops,* 4th Ed., pp. 172–175, 1995.

Rao et al., "Somatic Embryogenesis in Glume Callus Cultures," *Maize Genetics Cooperation Newsletter,* Vol. 60, 1986.

Songstad et al. "Effect of 1-Aminocyclopropate-1-Carboxylic Acid, Silver Nitrate, and Norbornadiene on Plant Regeneration from Maize Callus Cultures," *Plant Cell Reports,* 7:262–265, 1988.

Stuber et al., "Techniques and scoring procedures for starch gel electrophoresis of enzymes of maize *C. Zea mays, L.,*" *Tech. Bull.,* N. Carolina Agric. Res. Serv., Vol. 286, 1988.

Wan et al., "Efficient Production of Doubled Haploid Plants Through Colchicine Treatment of Anther-

Derived Maize Callus," *Theoretical and Applied Genetics*, 77:889–892, 1989.

What is claimed is:

1. Inbred corn seed of the corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

2. The inbred corn seed of claim 1, further defined as an essentially homogeneous population of inbred corn seed designated 87DIA4.

3. The inbred corn seed of claim 1, further defined as essentially free from hybrid seed.

4. An inbred corn plant produced by growing the seed of an inbred corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

5. Pollen of the plant of claim 4.

6. An ovule of the plant of claim 4.

7. An essentially homogeneous population of corn plants produced by growing the seed of an inbred corn plant designated 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

8. A corn plant having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

9. The corn plant of claim 8, further comprising a cytoplasmic factor conferring male sterility.

10. A tissue culture of regenerable cells of inbred corn plaint 87DIA4, wherein the tissue regenerates plants having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

11. The tissue culture of claim 10, wherein the regenerable cells are embryos, meristematic cells, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks, stalks, or protoplasts or callus derived therefrom.

12. A corn plant regenerated from the tissue culture of claim 10, having all the physiological and morphological characteristics of corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

13. An inbred corn plant cell of the corn plant of claim 4 having:

(a) an RFLP genetic marker profile in accordance with the profile shown in Table 8; or

(b) a genetic isozyme typing profile in accordance with the profile shown in Table 9.

14. The inbred corn plant cell of claim 13, having an RFLP genetic marker profile in accordance with the profile shown in Table 8.

15. The inbred corn plant cell of claim 13, having a genetic isozyme typing profile in accordance with the profile shown in Table 9.

16. The inbred corn plant cell of claim 13, having an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 8 and 9.

17. The inbred corn plant cell of claim 13, located within a corn plant or seed.

18. The inbred corn plant of claim 4 having:

(a) an RFLP genetic marker profile in accordance with the profile shown in Table 8; or

(b) a genetic isozyme typing profile in accordance with the profile shown in Table 9.

19. The inbred corn plant of claim 18, having an RFLP genetic marker profile in accordance with the profile shown in Table 8.

20. The inbred corn plant of claim 18, having a genetic isozyme typing profile in accordance with the profile shown in Table 9.

21. The inbred corn plant of claim 18, having an RFLP genetic marker profile and a genetic isozyme typing profile in accordance with the profiles shown in Tables 3 and 9.

22. A process of preparing corn seed, comprising crossing a first parent corn plant with a second parent corn plant, wherein said first or second corn plant is the inbred corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192, wherein seed is allowed to form.

23. The process of claim 22, further defined as a process of preparing hybrid corn seed, comprising crossing a first inbred corn plant with a second, distinct inbred corn plant, wherein said first or second corn plant is the inbred corn plant 87DIA4, a sample of the seed of said corn plant having been deposited under ATCC Accession No. 203192.

24. The process of claim 23, wherein crossing comprises the steps of:

(a) planting in pollinating proximity seeds of said first and second inbred corn plants;

(b) cultivating the seeds of said first and second inbred corn plants into plants that bear flowers;

(c) emasculating the male flowers of said first or second inbred corn plant to produce an emasculated corn plant;

(d) allowing cross-pollination to occur between said first and second inbred corn plants; and

(e) harvesting seeds produced on said emasculated corn plant.

25. The process of claim 24, further comprising growing said harvested seed to produce a hybrid corn plant.

26. Hybrid corn seed produced by the process of claim 23.

27. A hybrid corn plant produced by the process of claim 25.

28. The hybrid corn plant of claim 27, wherein the plant is a first generation (F_1) hybrid corn plant.

29. The corn plant of claim 8, further comprising a single gene conversion.

30. The corn plant of claim 29, wherein the single gene was stably inserted into a corn genome by transformation.

31. The single gene conversion of the corn plant of claim 29, where the gene is a dominant allele.

32. The single gene conversion of the corn plant of claim 29, where the gene is a recessive allele.

33. The single gene conversion corn plant of claim 29, where the gene confers herbicide resistance.

34. The single gene conversion of the corn plant of claim 29, where the gene confers insect resistance.

35. The single gene conversion of the corn plant of claim 29, where the gene confers resistance to bacterial, fungal, or viral disease.

36. The single gene conversion of the corn plant of claim 29, wherein the gene confers male sterility.

37. The single gene conversion of the corn plant of claim 29, where the gene confers waxy starch.

38. The single gene conversion of the corn plant of claim 29, where the gene confers improved nutritional quality.

39. The single gene conversion of the corn plant of claim 29, where the gene confers enhanced yield stability.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 5, at lines 46-47, please delete "Ear-Fresh Husk The color of the husks 1 to 2 weeks after pollination scored as Color: green, red, or purple" and substitute therefor --Ear-Fresh Husk Color: The color of the husks 1 to 2 weeks after pollination scored as green, red, or purple--.

In col. 5, at lines 56-57, please delete "Ear-Number Per The average number of ears per plant Stalk:" and substitute therefor --Ear-Number Per Stalk: The average number of ears per plant--.

In col. 5, at lines 58-59, please delete "Ear-Shank The average number of internodes on the ear shank. Internodes:" and substitute therefor --Ear-Shank Internodes: The average number of internodes on the ear shank--.

In col. 6, at lines 51-53, please delete "Kernel-Aleurone The color of the aleurone scored as white, pink, tan, brown, Color: bronze, red, purple, pale purple, colorless, or variegated" and substitute therefor --Kernel-Aleurone Color: The color of the aleurone scored as white, pink, tan, brown, bronze, red, purple, pale purple, colorless, or variegated--.

In col. 6, at lines 57-58, please delete "Kernel-Endosperm The color of the endosperm scored as white, pale yellow, or Color: yellow" and substitute therefor --Kernel-Endosperm Color: The color of the endosperm scored as white, pale yellow, or yellow--.

In col. 6, at lines 59-60, please delete "Kernel-Endosperm The type of endosperm scored as normal, waxy, or opaque. Type:" and substitute therefor --Kernel-Endosperm Type: The type of endosperm scored as normal, waxy, or opaque--.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 6, at lines 65-66, please delete "Kernel-Number Per The average number of kernels in a single row. Row:" and substitute therefor --Kernel-Number Per Row: The average number of kernels in a single row--.

In col. 7, at lines 1-3, please delete "Kernel-Pericarp The color of the pericarp scored as colorless, red-white crown, tan, Color: bronze, brown, light red, cherry red, or variegated" and substitute therefor -- Kernel-Pericarp Color: The color of the pericarp scored as colorless, red-white crown, tan, bronze, brown, light red, cherry red, or variegated--.

In col. 7, at lines 4-6, please delete "Kernel-Row The direction of the kernel rows on the ear scored as straight, Direction: slightly curved, spiral, or indistinct (scattered)" and substitute therefor --Kernel-Row Direction: The direction of the kernel rows on the ear scored as straight, slightly curved, spiral, or indistinct (scattered)--.

In col. 7, at lines 30-33, please delete "Leaf-Longitudinal A rating of the number of longitudinal creases on the leaf surface 1 Creases: to 2 weeks after pollination. Creases are scored as absent, few, or many" and substitute therefor --Leaf-Longitudinal Creases: A rating of the number of longitudinal creases on the leaf surface 1 to 2 weeks after pollination. Creases are scored as absent, few, or many--.

In col. 7, at lines 34-36, please delete "Leaf-Marginal A rating of the waviness of the leaf margin 1 to 2 weeks after Waves: pollination. Rated as none, few, or many" and substitute therefor --Leaf-Marginal Waves: A rating of the waviness of the leaf margin 1 to 2 weeks after pollination. Rated as none, few, or many--.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 7, at lines 40-43, please delete "Leaf-Sheath A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks Anthocyanin: after pollination, scored as absent, basal-weak, basal-strong, weak or strong" and substitute therefor --Leaf-Sheath Anthocyanin: A rating of the level of anthocyanin in the leaf sheath 1 to 2 weeks after pollination, scored as absent, basal-weak, basal-strong, weak or strong--.

In col. 7, at lines 44-46, please delete "Leaf-Sheath A rating of the pubescence of the leaf sheath. Ratings are taken 1 Pubescence: to 2 weeks after pollination and scored as light, medium, or heavy" and substitute therefor --Leaf-Sheath Pubescence: A rating of the pubescence of the leaf sheath. Ratings are taken 1 to 2 weeks after pollination and scored as light, medium, or heavy--.

In col. 8, at lines 19-21, please delete "Stalk-Brace Root The color of the brace roots observed 1 to 2 weeks after pollination Color: as green, red, or purple" and substitute therefor --Stalk-Brace Root Color: The color of the brace roots observed 1 to 2 weeks after pollination as green, red, or purple--.

In col. 8, at lines 27-29, please delete "Stalk-Internode The direction of the stalk internode observed after pollination as Direction: straight or zigzag" and substitute therefor --Stalk-Internode Direction: The direction of the stalk internode observed after pollination as straight or zigzag--.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In col. 8, at lines 30-31, please delete "Stalk-Internode The average length of the internode above the primary ear. Length:" and substitute therefor --Stalk Internode Length: The average length of the internode above the primary ear--.

In col. 8, at lines 35-36, please delete "Stalk-Internode With The average number of nodes having brace roots per plant. Brace Roots:" and substitute therefor --Stalk-Internode With Brace Roots: The average number of nodes having brace roots per plant--.

In col. 8, at lines 65-66, please delete "Tassel-Branch The average number of primary tassel branches. Number:" and substitute therefor --Tassel-Branch Number: The average number of primary tassel branches--.

In col. 9, at lines 7-9, please delete "Tassel-Peduncle The average length of the tassel peduncle, measured from the base of the flag leaf to the base Length: of the bottom tassel branch" and substitute therefor --Tassel-Peduncle Length: The average length of the tassel peduncle, measured from the base of the flag leaf to the base of the bottom tassel branch--.

In col. 13, at line 26, delete "3AZA1" and substitute therefor --AQA3--.

In col. 14, at line 52, delete "87DIA114" and substitute therefor --87DIA4--.

In col. 30, at line 4, , delete "DEKALB Plant Genetics" and substitute therefor --DEKALB Genetics Corporation--.

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At col. 17, in Table 5 delete all rows under "4. EAR" and ending with "Endosperm Color" and substitute therefor the following rows --

4. EAR				
Silk Color	Pink	Pink	—	Red
Number Per Stalk	1.0	1.0	1.0	1.4
Position (attitude)	Upright	—	—	Upright
Length cm.	14.6	16.0	14.6	15.6
Shape	Semi-conical	Semi-conical	Semi-conical	Semi-conical
Diameter cm.	4.0	3.8	4.0	3.9
Weight gm.	104.9	100.6	103.2	107.6
Shank Length cm.	10.3	14.1	10.1	9.6
Husk Bract	Short	Short	Short	Short
Husk Cover cm.	6.4	2.5	4.4	3.7
Husk Color Fresh	Green	Green	Green	Green
Husk Color Dry	Buff	Buff	Buff	Buff
Cob Diameter cm.	2.3	2.4	2.3	2.1
Cob Color	Red	—	Red	Red
Shelling Percent	87.7	80.6	89.0	83.3

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 5,936,145

DATED : August 10, 1999

INVENTOR(S) : Peter J. Bradbury

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

5. KERNEL				
Row Number	14.6	14.8	16.3	15.3
Number Per Row	32.1	27.1	29.3	29.7
Row Direction	Curved	Curved	Curved	Curved
Type	Dent	—	Dent	—
Cap Color	Yellow	Yellow	Yellow	Yellow
Side Color	Deep Yellow	—	Orange	
Length (depth) mm.	11.1	9.4	10.9	10.3
Width mm.	7.8	8.0	7.4	7.8
Thickness	3.9	5.2	4.4	4.2
Weight of 1000K gm.	269.0	252.4	233.0	247.8
Endosperm Type	Normal	Normal	Normal	Normal
Endosperm Color	Yellow	Yellow	Yellow	Yellow—.

Signed and Sealed this

Twenty-seventh Day of February, 2001

Attest:

Nicholas P. Godici

NICHOLAS P. GODICI

Attesting Officer

Acting Director of the United States Patent and Trademark Office